CHAPTER 5

FREQUENCY OF HAEMOGLOBIN STRUCTURAL DEFECT (HAEMOGLOBINOPATHIES) IN SAUDI ARABIA

5.1 Introduction

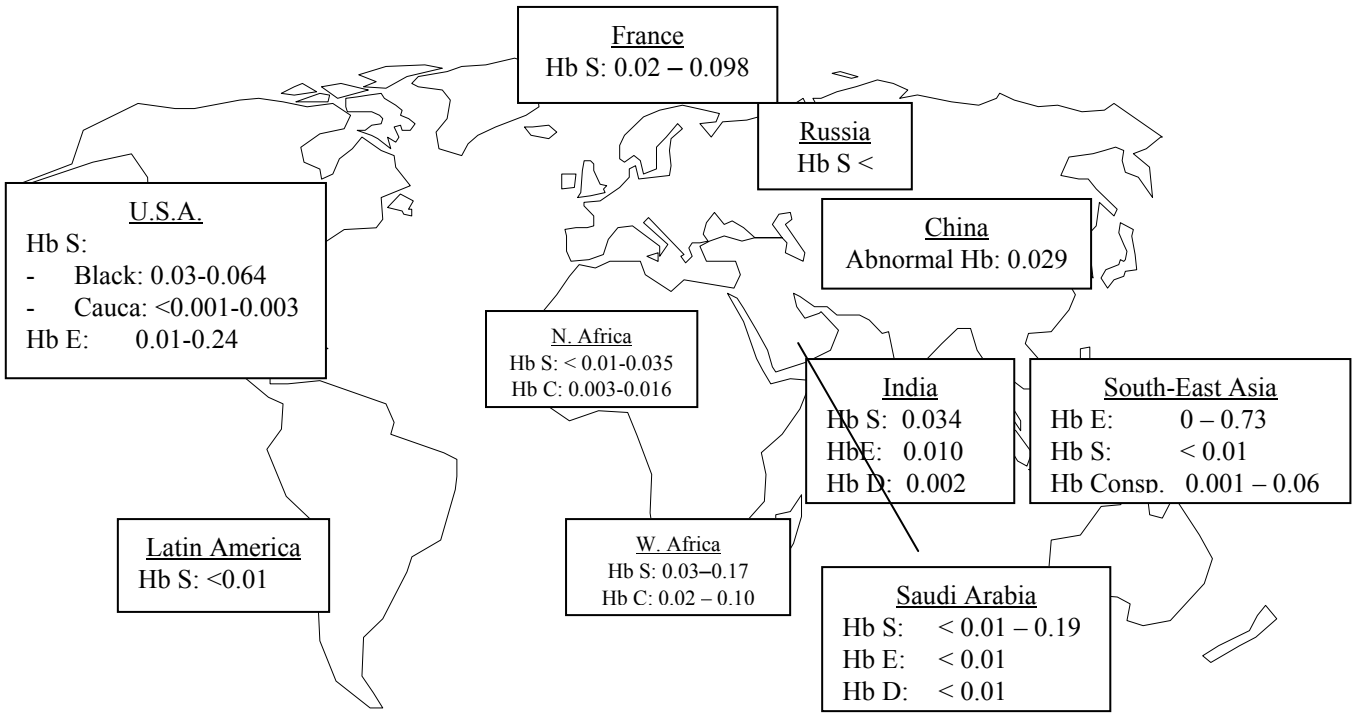
The structural defects in haemoglobin molecule result from mutations in the exons of the globin genes and produce conditions which are broadly classified as `haemo-globinopathies'. This heterogenous group of genetic disorders along with biosynthetic defects affecting haemoglobin synthesis constitutes the most widely distributed and most extensively investigated single gene disorders in the world populations (Weatherall and Clegg, 1981; Bowman, 1983; Beutler, 1978, 1990; Winter, 1987).

5.2 World distribution of haemoglobinopathies

The most frequently identified haemoglobinopathies are those resulting from inheritance of Hb S. For a long time, there existed a misconception that Hb S is limited only to the Negro race, however, later extensive studies revealed the occurrence of Hb S in non-Negro population in India, Southern Italy, Northern Greece, Sicily, Southern Turkey, Saudi Arabia and North Africa (Livingstone 1967; Bowman, 1983). In addition, as a result of population admixtures and movements the Hb S is also identified, though at lower frequencies in the European countries, the Americas and in Canada. Within each country differences in the gene frequency are reported in different areas and in different racial groups and tribes. The most well investigated are the tribes in Africa, living in the same locality but with significant difference in the gene frequency of Hb S gene. The Bantu speaking tribes , for example, have a significantly higher Hb S gene frequency compared to the Hamitic speaking tribes in Uganda and Tanzania. Figure 5.1 presents a rough guide to the Hb S gene frequency in the different parts of the world.

The maintenance of high frequency of Hb S in certain areas of the world has led to

Figure 5.1: A rough guide to the frequency of Hb S gene in the world



the suggestion that the heterozygous for the Hb S gene have some advantage over normal homozygous (Hb AA) and sickle cell disease patients and are, therefore, able to survive and reproduce. Thus as a result of "balanced polymorphism" the Hb S gene has been maintained at relatively constant levels in populations. It was as early as in 1952 when Mackey and Vivarelli suggested that the environmental factors maintaining Hb S gene is malaria (Mackey & Vivarelli, 1952). Several in vivo and in vitro studies have since confirmed the "malaria hypothesis" and a close correlation is demonstrated between malaria endemicity and the Hb S gene (Allison 1961, Livingstone 1983; Gelpi, 1967). There is evidence that the Hb S heterozygotes have a natural resistance against malaria and are therefore able to survive during the childhood when malaria caused by *Falciparum malaria* is most critical and fatal. The other frequently identified Hb structural variants include Hb C, Hb E, Hb D Punjab and Hb O Arab. Each of these variants occurs at a high frequency in some populations, where polymorphic levels have been reached, while in other areas sporadic cases have been reported. In addition, unlike the correlation between Hb S gene frequency and malaria, no other similar environmental factor has yet been identified that could explain the high frequency of these haemoglobins in some areas.

Haemoglobin C trait frequency has been reported as high as 20% in Upper Volta and in northern Ghana. In other West African countries, the prevalence ranging from 1->10% has been reported. The movement of population from Africa to the Caribbean, North America and France has resulted in polymorphic gene frequency of Hb C in black populations in these areas. Haemoglobin E, a structural Hb variant, that is also thalassaemic, is confined largely to the population of South-East Asia, where frequencies

ranging from 0 to 0.73 have been reported in different countries. Haemoglobin E is considered as a hall mark of South-East Asia just as Hb S is for Africa. However, cases have been reported from China, Australia, New Zealand, Canada and France. Generally these are the families of the Southeast Asians and Indians who have migrated and settled in these countries.

Haemoglobin D Punjab is largely confined to the Indian population where almost 1% of the Punjabi Sikhs have Hb D. In Pakistan, Southeast Asia, Northern China, the Middle East, Australia, New Zealand, America and Britain, Hb D Punjab has been reported.

Finally, Hb O Arab occurs at polymorphic frequency in the northern part of east and west Africa. It is also reported in Sudan, Jamaica, Kenya, the United States, Greece and the European countries. The highest gene frequency is reported in Bulgaria.

5.3 Haemoglobinopathies in Saudi Arabia

Haemoglobinopathies due to structural abnormalities of haemoglobin, were first recognized in Saudi Arabia in 1963 by Lehmann and co-workers during a screening programme in the eastern province of Saudi Arabia (Lehmann et al, 1963). Thereafter, Gelpi reported the presence of sickle cell gene in Saudis living in the oasis population of Al-Qateef and Al-Hasa (Gelpi, 1967). The author also observed that the sickle cell anaemia was mild in this population and suggested that other coexisting genetic abnormalities, such as G-6-PD deficiency or the thalassaemias or other abnormal haemoglobin variants, ameliorate the clinical presentation of sickle cell anaemia, thus producing a benign or mild form of the disease (Gelpi, 1970). At about the same time,

Weatherall and co-workers described a mild form of sickle cell disease in a patient with sickle cell homozygosity and α-thalassaemia gene and termed the condition as "a new sickling disorder" (Weatherall et al, 1969). Subsequently, the benign nature of sickle cell disease in the Saudis from the Al-Qateef and Al-Hasa oases was confirmed and the presence of a high percentage of haemoglobin F was considered as an ameliorating factor (Pembrey et al, 1975, 1978,; Perrine et al, 1972; Wood et al, 1980). The homozygous sickle cell anaemia patients in the eastern province were easily distinguishable from those of African origin by the mildness of clinical manifestations and the associated high Hb F level (Perrine et al, 1978; Pembrey et al, 1978; Perrine et al, 1972). The lower incidence of vaso-occlusive complications, persistence of splenic functions, lower morbidity due to other complications and lower risk during pregnancy were all attributed to elevated Hb F in the Saudi patients. However, later studies revealed a mild sickle cell anaemia even in the absence of elevated levels of Hb F (El-Hazmi, 1976, 1979, 1980).

5.3.1. Sickle cell gene in Saudi Arabia

Studies conducted in different regions of Saudi Arabia during the late 1970s and early 1980s revealed the presence of Hb S and other red cell genetic defects in several regions of the country (El-Hazmi 1976; 1979; 1980). Significant differences were reported in the different areas of the country and even within the same areas Hb S gene frequency was found to be significantly variable (El-Hazmi 1976, 1979, 1980, 1982).

In 1980, King Abdulaziz City for Science and Technology financed a project on the study of Natural History of Sickle Cell Disease in the Eastern Province population. This study was based on the screening of neonates and follow-up of the sickle cell disease

patients during childhood. This study reported a gene frequency of Hb S of 5% to 27% of newborn in Al-Khober, Dammam and Qateef (Al-Awamy et al, 1986, 1991).

In 1982, we initiated the detailed screening of the Saudi population in the different regions of the country. Several abnormal haemoglobins were identified. These are listed in Table 5.1. Only sickle cell haemoglobin (Hb S) was found to be polymorphic while all othersoccurred in a few cases and were rare variants (frequency <0.01) (El-Hazmi, 1983, 1985a,b, 1987a,b,c, 1992).

Haemoglobin S was identified by electrophoresis at alkaline and acid pH and at the DNA level using the restriction endonuclease Mst II. Mst II produces a 1.15 Kb fragment in Hb AA, 1.15 Kb and 1.35 Kb in Hb AS and 1.35 Kb in Hb SS cases as shown in Figures 5.2 and 5.3. The prevalence of Hb S heterozygotes and homozygotes in each area is presented in Table 5.2. The Hardy-Weinburg Equilibrium was applied and in each region the number of observed cases were found to be significantly higher compared to the expected number (Table 5.3).

The Hb S gene frequency was calculated in each regions and the results are presented in Fig 5.4 and Table 5.4. Applying the ANOVA program the results showed that the difference in the frequency in different regions was statistically significant ($P< 0.01$).

The gene frequency in the populations is determined by the Hardy-Weinberg principle, according to which genotype frequencies remain constant from generation to generation. It also provides an equation for the equilibrium frequencies of the genotypes and their respective genes. If the 2 alleles (i.e. genes that occupy the same loci on a pair of homologous chromosome) occur at a frequency of 'p' and 'q', respectively, then according

to Hardy-Weinberg equilibrium the expected proportion of the respective genotype in a population will be given by:

$$p^2 + 2pq + q^2 = 1$$

Table 5.1: Mutant haemoglobins identified in Saudi Arabia

Mutant Haemoglobins identified	Status	Reference
• Haemoglobin S	Polymorphic	This study
• Haemolgobin C	Rare	" "
• Haemoglobin D-Punjab	Rare	" "
• Haemoglobin O-Arab	Rare	El-Hazmi & Lehmann 1980
• Haemoglobin E	Rare	" " "
• Haemoglobin H	Rare	" " "
• Haemoglobin G	Rare	" " "
• Hb F-Dammam	Rare	Al-Awamy et al 1985a
• Hb Qatif	Rare	-
• Hb Handsorth	Rare	Al-Awamy et al 1985b
• Hb Riyadh	Rare	El-Hazmi 1976-1977
• Hb Setif	Rare	Al-Awamy et al 1985c

Figure 5.2: DIAGNOSIS OF SICKLE CELL MUTATION USING Mst II

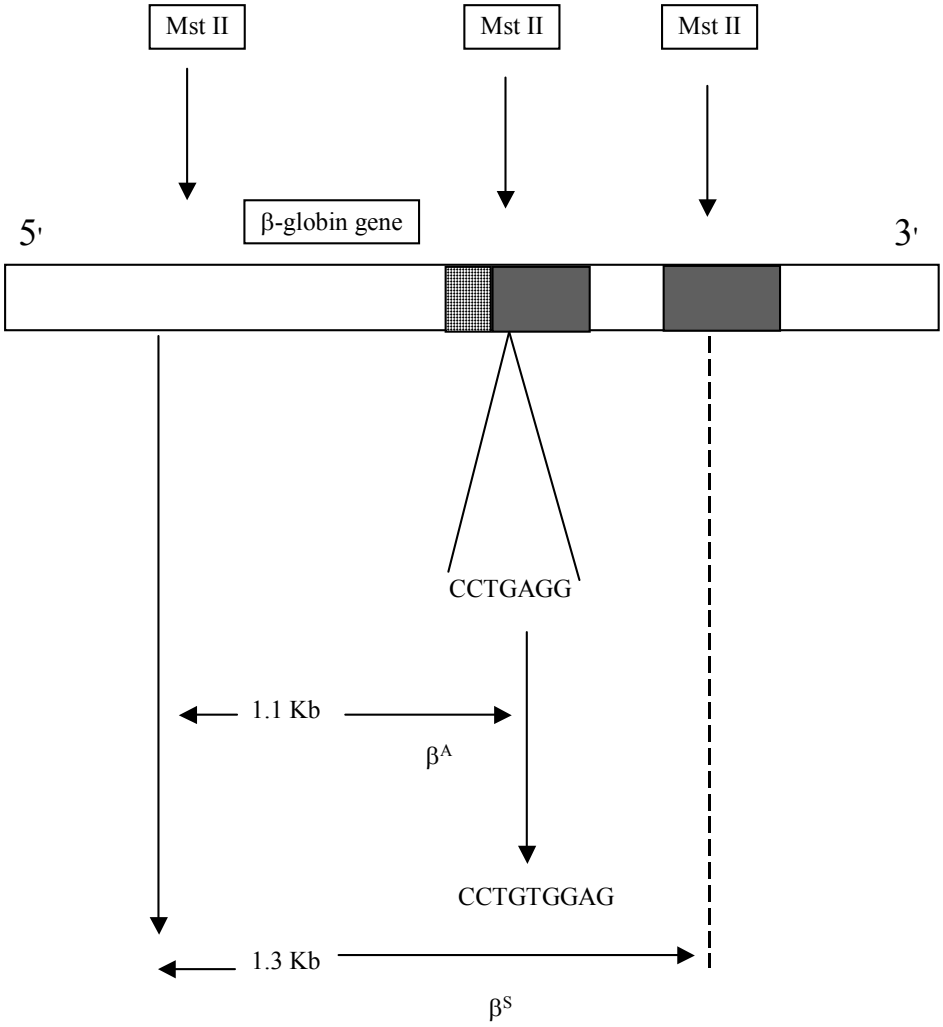


Figure 5.3: Autoradiograph of fragments carrying the β-globin gene produced by Mst II digestion

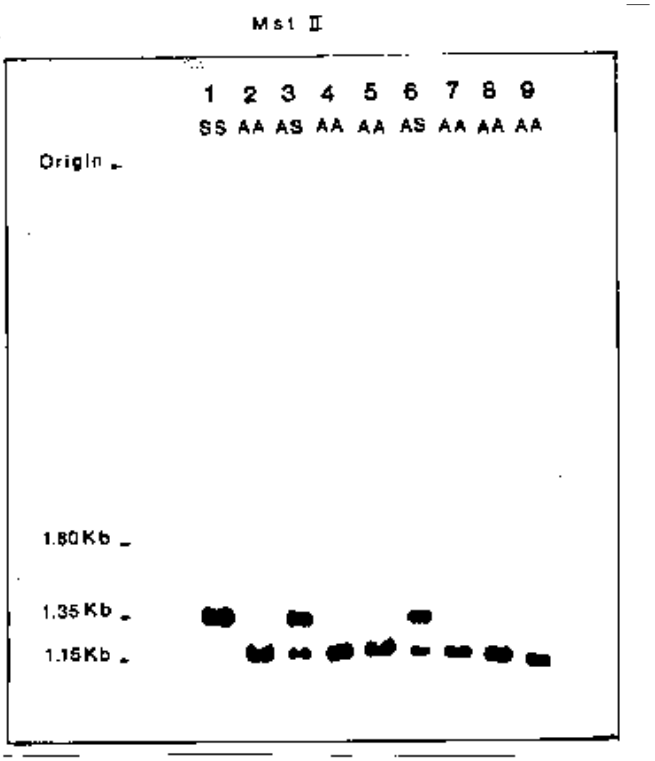


Table 5.2: Prevalence of Hb S heterozygotes and homozygotes in different regions of Saudi Arabia

Province	Area	No. Investi-gated	No. of AS	Prev. (%)	No. of SS	Prev. (%)
Eastern	Al-Qateef	962	249	25.88	43	4.47
	Al-Hafouf	1490	326	21.9	61	4.1
	Dammam	401	32	9.0	5	1.2
Central	Riyadh	3994	44	1.1	2	0.05
	Qaseem	1015	2	0.2	0	0
	Buraida	1232	4	0.32	0	0
	Al-Russ	653	0	0	0	0
	Al-Unaiza	355	0	0	0	0
	Al-Mesnab	287	0	0	0	0
	Bkaria	159	0	0	0	0
	Sulayel	1362	29	2.13	5	0.37
	Hafar Al-Batin	814	2	0.25	2	0.25
	Wadi-i-Dawasir	229	3	1.31	0	0
Western	Al-Ula	409	50	12.22	8	1.96
	Khaiber	1016	138	13.6	15	1.5
	Yanbu	1095	30	2.74	2	0.18
	Makkah	877	38	4.33	5	0.57
	Qunfuda	823	147	17.86	21	2.55
	Bisha	933	89	9.54	33	3.54
	Najran	1860	44	2.4	12	0.65
	Jaizan	1466	283	19.3	10	0.7
	Sabya	642	104	16.19	5	0.77
	Samtah	349	29	8.30	0	0
	Abu Areesh	380	62	16.3	2	0.52
	Farasan	176	0	0	0	0
	Baish	278	27	9.71	4	1.44
	Fifa	172	44	25.5	4	2.325
	Al-Baha	1071	30	2.80	5	0.47
	Mahayel	645	88	13.64	34	5.27
	Abha	1108	216	19.49	34	3.07
	Majarda	272	55	20.2	6	2.20
Northern	Hail	1646	11	0.66	0	0
	Tabuk	888	27	3.04	0	0
	Arar	670	8	1.19	0	0
	Qurayat	46	0	0	0	0
	Al-Jouf	280	0	0	0	0

Table 5.3: Expected and observed number of Hb S homozygotes in different regions of Saudi Arabia

Province	Area	Observed	Expected*
Eastern	Al-Qateef	43	20.5
	Al-Hafouf	61	22.0
	Dammam	5	0.59
Central	Riyadh	2	0.122
	Qaseem	0	0
	Buraida	0	0
	Al-Russ	0	0
	Al-Unaiza	0	0
	Al-Mesnab	0	0
	Bkaria	0	0
	Sulayel	5	0.16
	Hafr-Al-Batin	2	0.0012
	Wadi-i-Dawasir	0	0.0099
Western	Al-Ula	8	1.72
	Khaiber	15	5.3
	Yanbu	2	0.211
	Makkah	5	0.43
	Qunfuda	21	7.79
	Bisha	33	2.33
	Najran	2	0.27
	Jaizan	10	16.4
	Sabya	5	4.86
	Samtah	0	0.651
	Abu Areesh	2	2.9
	Farasan	0	0
	Baish	4	0.720
	Fifa	4	3.57
	Al-Baha	5	0
	Mahayel	34	0.720
	Abha	34	3.57
	Majarda	6	3.36
Northern	Hail	0	0
	Tabuk	0	0
	Arar	0	0
	Qurayat	0	0
	Al-Jouf	0	0

* Calculated using Hardy Weinburg equilibrium

Figure 5.4: Sickle Cell (Hb S) Gene Frequency in some regions of Saudi Arabia

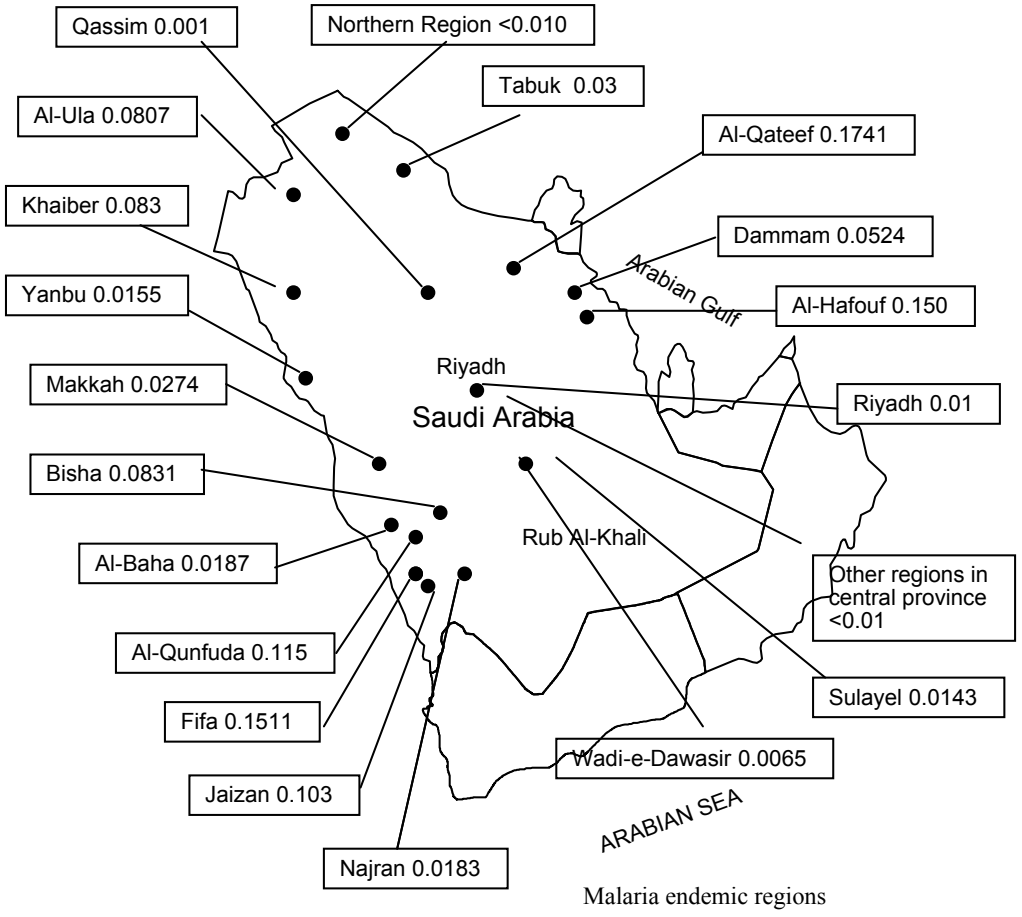


Table 5.4: Hb S gene frequency in different regions of Saudi Arabia

Province	Area	Hb S gene Frequency
Eastern	Al-Qateef	0.1741
	Al-Hafouf	0.150
	Dammam	0.0524
Central	Riyadh	0.006
	Qaseem	0.001
	Buraida	0.0016
	Al-Russ	0
	Al-Unaiza	0
	Al-Mesnab	0
	Bakaria	0
	Sulayel	0.0143
	Hafr-Al-Batin	0.0037
	Wadi-i-Dawasir	0.0065
Western	Al-Ula	0.0807
	Khaiber	0.083
	Yanbu	0.0155
	Makkah	0.0274
	Qunfuda	0.1148
	Bisha	0.0831
	Najran	0.0183
	Jaizan	0.103
	Sabya	0.0888
	Samta	0.0415
	Abu-Areesh	0.0868
	Farasan	0
	Baish	0.0629
	Fifa	0.1511
	Al-Baha	0.0187
	Mahayel	0.1209
	Abha	0.1281
	Majarda	0.1231
Northern	Hail	0.0033
	Tabuk	0.0304
	Arar	0.0059
	Qurayat	0
	Al-Jouf	0

The Hardy-Weinberg equilibrium is applicable to most populations when the mating is random, gene flow and drift is limited and selection does not occur. However, in presence of non-random mating, such as a high rate of consanguinity, or selection such as selection of Hb S heterozygotes in the presence of falciparum malaria (i.e. relative fitness of the genotype), or gene flow (i.e. changes in gene frequency due to population movement) or gene drifts (i.e. changes in gene frequency due to unknown causes), the Hardy-Weinberg equilibrium is disturbed and the number of homozygous cases identified in a population may be more than the expected cases.

This difficulty was faced during our studies on the Saudi population. Every area investigated had Hb SS cases (observed) more than the expected Hb SS cases calculated by applying the Hardy Weinberg equilibrium. This can be explained as follows:

i) The rate of consanguinity and other forms of intermarriages is extremely high in the Saudi population (over 50%). Marriages generally occur between members of the same family or within the members of the same tribe and the communities in each area are extremely closed. Thus the mating is highly non-random and this affects Hardy Weinberg equilibrium.

ii) Malaria has been or is endemic in the regions where the Hb S gene occurs in the Saudi populations. Since the Hb S heterozygotes have resistance against malaria, they survive more compared to the normal (Hb AA) or homozygous Hb SS and hence there is a higher proportion of Hb AS mating, this increases the chances of Hb SS offspring.

iii) The homozygous Hb SS disease is relatively mild particularly in the eastern

province of Saudi Arabia and the Hb SS cases survive to adult life, marry and have offspring.

iv) Gene flow occurs from villages and small town and the healthy individuals are moving out to big cities while several of those who remain have the abnormal gene and thus the gene pool remains high. (Gene flow could also be the mechanism by which the abnormal genes have reached the non-malaria endemic regions within Saudi Arabia).

The Hb S gene was encountered in all areas screened in Saudi Arabia. The Hb S gene frequency was the highest in Al-Qateef and Al-Hafouf in the Eastern Province of Saudi Arabia, followed by Mahayel, Abha, Al-Qunfuda and Jaizan in South-western province. In the North-western province, the frequency of Hb S gene was around 0.08 in Khaiber and Al-Ula. Generally, the non-malaria endemic regions such as the Central Province and the Northern Province, had a very low frequency of Hb S gene. Past or present malaria endemic regions have a high frequency of Hb S gene. However, in the malaria endemic regions there were a few exceptions where lower Hb S gene frequencies were encountered in Al-Baha, Najran and Yanbu which have been malaria endemic areas in the past.

5.3.2 Other abnormal haemoglobins in Saudi Arabia

In addition to the Hb S, other abnormal haemoglobins were identified in Saudi Arabia but only as sporadic cases and none exhibit polymorphism in any of the areas of Saudi Arabia. The mutant haemoglobins identified are listed in Table 5.1. The Hb C is a β-globin chain variant with a single point mutation in the codon 6 of the β-globin gene on

chromosome 11 which results in the substitution of an acidic amino acid, glutamic acid, by basic amino acid, lysine. The electrophoretic mobility of Hb C is significantly different from Hb A and both can be separated on electrophoresis at alkaline and acid pH. The highest frequencies of Hb C are reported in certain parts of West Africa and in some regions the Hb C prevalence in as high as 21.4%. However, sporadic cases are reported in other areas. No correlation could be demonstrated between malaria endemicity and Hb C gene frequency.

Cases of Hb C were identified in different regions. Haemoglobin E was identified in several cases as Hb AE (20 cases; frequency < 0.001), in different parts of the country. No cases of homozygous Hb E were identified.

5.3.3 The origin of abnormal haemoglobin variants

Considerable interest has been devoted to the investigation of the origin of Hb S. The earlier investigators believed that the Hb S was limited to the Negro race, but later extensive population studies showed the wide spread distribution of the Hb S gene. Whether Hb S gene mutation occurred at one place and then spread to the different parts of the world, or whether the same mutation occurred in different areas has been the subject of considerable debate. Several of the earlier workers including Lehmann (1954a,b), Huntman (Lehman and Huntman, 1974) and Gelpi (1973) have favoured the single mutation theory. It was suggested by Lehmann (1954a, b) that during the neolithic times, the Arabian Peninsula was a fertile land and the mutation occurred in the population group living in this area. As the climatic conditions started to change and the area slowly lost its fertility changing to a desert, the population started to migrate in all directions. As they

moved to India, Equatorial Africa and Saudi Arabia, the spread of the Hb S gene took place. This hypothesis was supported by the fact that the gene frequency declines from the east to west Africa (Lehmann and Huntsman, 1974). Gelpi (1973) on the other hand, suggested that the Hb S gene originated in the Equatorial Africa and from there it spread to different parts of the world including Arabia, India and the Mediterranean region during the slave trade days (Kamel and Awny, 1965).

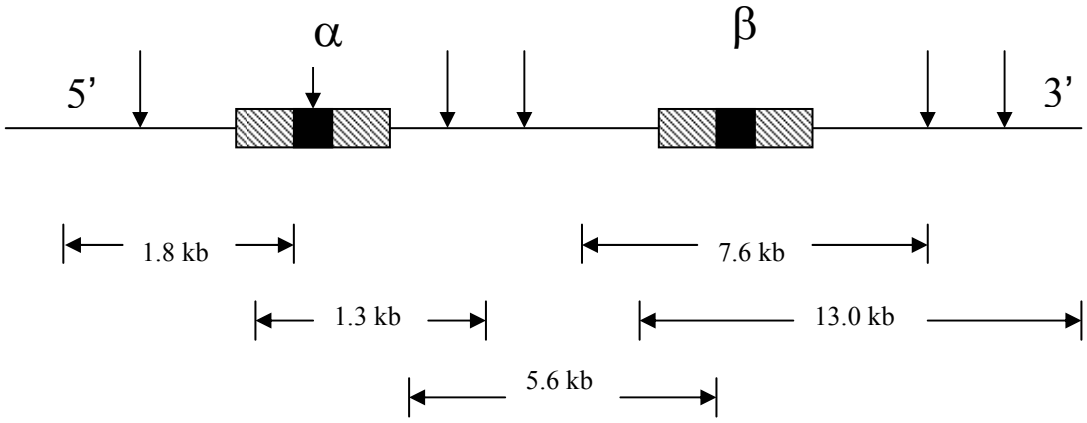
More recently, since the advent of the techniques of molecular biology and the study of β^S globin gene restriction fragment length polymorphisms (RFLPs), the studies at the gene level have favoured the multiple mutation theory.

5.3.4 The Hpa I Polymorphism

The restriction endonuclease Hpa I restricts the ββ-globin gene on chromosome 11 and produces a 7.6 kb fragment. In 1978 Kan and Dozy, first reported a polymorphism and showed that the β^S globin gene was linked to a polymorphic site 3' end of the β-globin gene thus producing a 13.0 kb fragment. In the African population, the β^S was found to be relatively tightly linked to the β^S globin gene and in Hb S homozygotes the frequency of 13.0 kb fragment was reported as 0.87 (Kan and Dozy, 1978). The 13.0 Kb fragment was also found to be linked to the β^S gene (Feldenzer et al, 1979). More detailed studies, however, revealed a complex picture where it was showed that though in parts of Western Africa, black Americans of West African origin in Algeria, Morocco, Togo, and Sicily, the β^S was mainly linked to the 13.0 kb fragment, while in other parts of the world significant variations were found (Kan & Dozy, 1980). Reports showed that β^S is also linked to 7.6 kb fragment in Gabon, Kenya, India and the Ivory Coast. Later, the β^S and β^A genes were

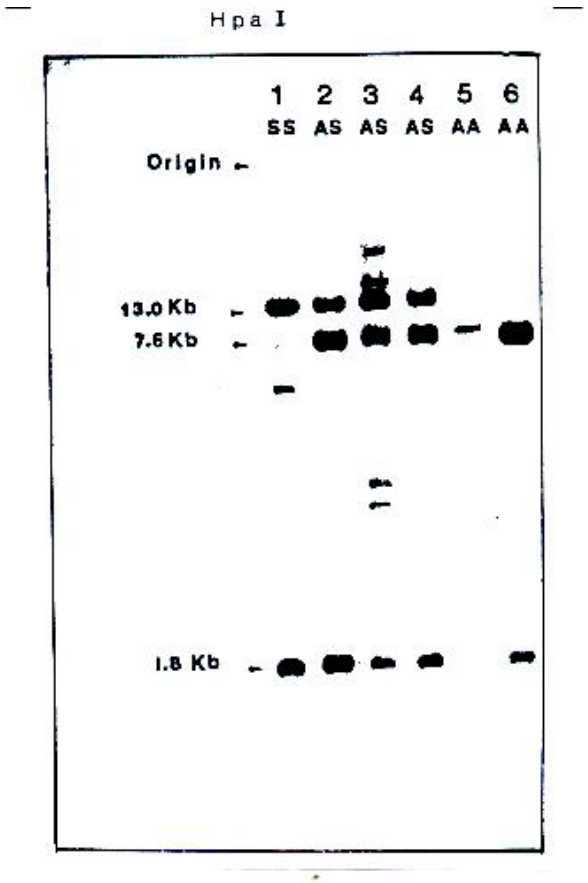
found to be linked to a 7.0 kb and 5.6 kb Hpa I fragments in addition to 13.0 Kb and 7.6 Kb fragments (Figures 5.5 and 5.6). These results indicated that the β^S mutation occurred independently on different chromosomes bearing different

Figure 5.5: β-globin gene polymorphism using restriction endonuclease Hpa I



(Each arrow pointing downward (↓) indicates a Hpa I site. The polymorphic site in the one 3' to the β-globin gene, which results in the production of fragments of different sizes carrying the β=globin gene)

Figure 5.6: Autoradiograph of fragments carrying the β-globin gene produced by Hpa I digestion



polymorphic sites of restriction endonucleases or that the polymorphic sites were produced as a result of mutations on chromosomes bearing β^S gene of single origin. Most opinions favour the first possibility and suggest the occurrence of β^S mutation on different chromosomes in different locations.

5.3.5 Hpa I polymorphism in Saudi Arabia

During our investigations we investigated Hpa I polymorphism in normal (Hb AA), Hb S heterozygotes (Hb AS) and Hb S homozygotes (Hb SS) from different regions of Saudi Arabia (Figures 5.5 and 5.6). In contrast to the earlier reports that β^S is linked mainly to the 7.6 kb fragment in the Saudis (Kan and Dozy, 1980), our results showed significant differences in the results from the different parts of Saudi Arabia (Figure 5.7). In the eastern province the β^S was linked mainly to the 7.6 kb fragment, while in the while in the western province it was linked mainly to the 13.0 kb Hpa I fragment. Cases with β^S and β^A linked to 5.6 and 7.0 kb fragments were also identified in Saudis, but at a considerably lower frequency. Thus confirming the reports that Hpa I exhibits extensive polymorphism at site 3' to the β-globin gene. Our results suggest that in Saudi Arabia there are two different origins of the sickle cell gene, where the Eastern Saudi Arabia shows similar pattern to the Asian neighbours while the Western Saudi Arabia resemble their African neighbours in the Hpa I polymorphism pattern (El-Hazmi, 1986; El-Hazmi and Warsy, 1987; El-Hazmi et al, 1986).

5.3.6 The beta globin gene haplotypes

Further analysis of the β-globin gene cluster on chromosome 11 using several restriction endonucleases revealed a number of restriction fragment length polymorphic

Figure 5.7: The Hpa I polymorphism in Saudi Arabia

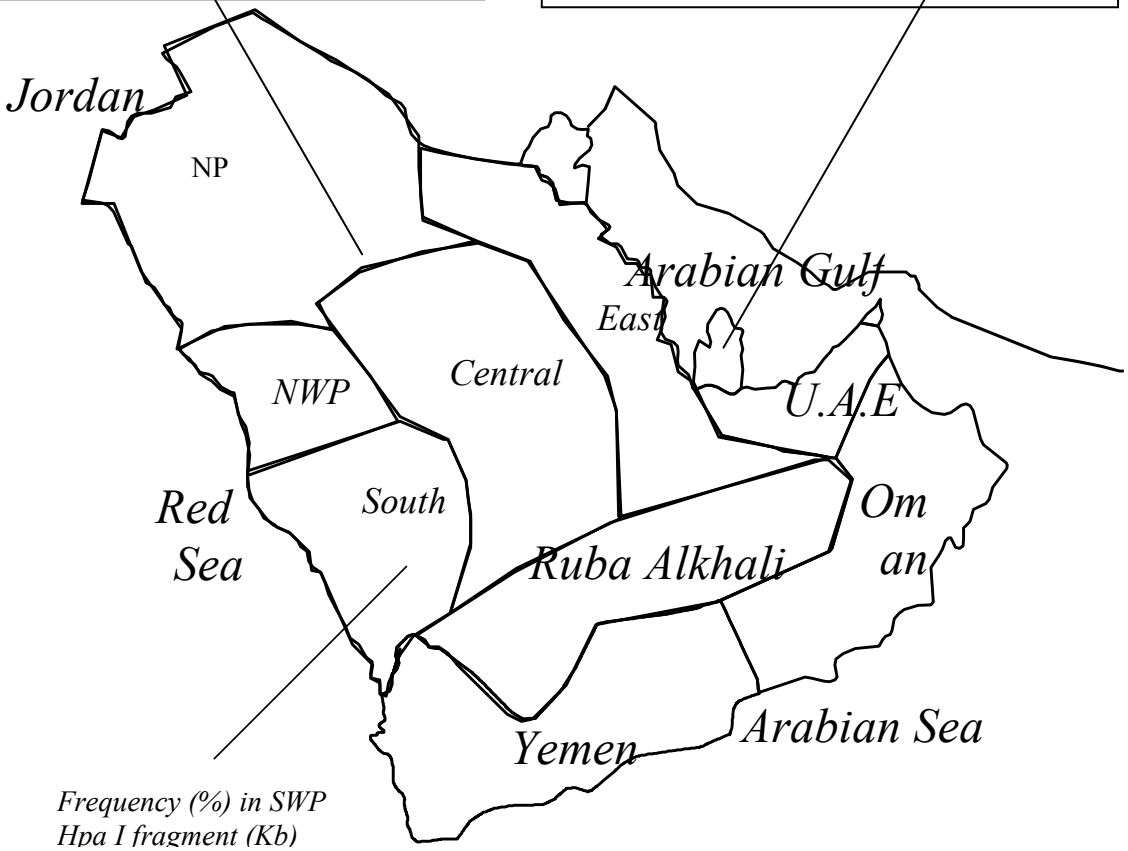
`

Frequency (%) in NWP
Hpa I fragment (kb)

Hb genotype	13.0	7.6	7.0	5.6
AA	16.7	80.9	-	-
AS	51.3	48.2	-	-
SS	100		-	-

Frequency (%) in EP
Hpa I fragment (kb)

Hb genotype	13.0	7.6	7.0	5.6
AA	7.1	88.6	0.43	-
AS	6.3	92.8	0.8	-
SS	12.5	87.5	-	-



Frequency (%) in SWP
Hpa I fragment (Kb)

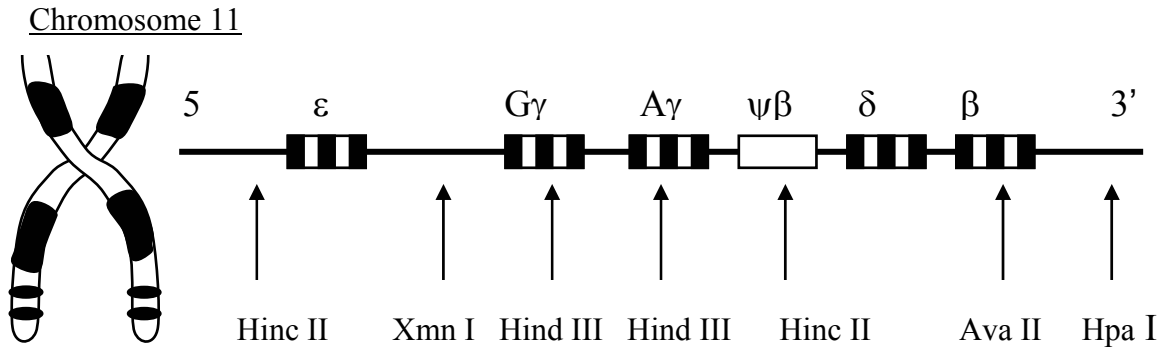
Hb genotype	13.0	7.6	7.0	5.6
AA	3.8	92.3	3.8	-
AS	50.0	50.0	-	-
SS	75.0	25.0	-	-

sites. Using the plus (+) sign to indicate the presence and a minus (-) sign to indicate the absence of the restriction endonuclease sites specific β-globin gene haplotypes were constructed. Figure 5.8 presents the schematic presentation of the β-globin gene cluster and the approximate location of the restriction sites of the restriction endonucleases, Hinc II, Hind III, Ava II, Xmn I and Hpa I and Table 5.5 presents the size of fragments generated in presence or absence of the polymorphic site. The patterns constructed were referred to the β-globin gene haplotypes. A large number of different haplotype pattern were obtained in association with β^A, β^S and β-Thalassaemia (Antonarakis et al, 1982, 1985). Four main haplotypes were identified and these were named as the `Benin haplotype', the `Bantu haplotype', the `Senegal haplotype' and the `Saudi-Indian haplotype'. These haplotype patterns are presented in Figure 5.9. The haplotypes were found to be confined largely to specific populations and indicated four main foci of the origin of the sickle cell gene. Three of these (Senegal, Benin and Bantu) are believed to have originated in Africa, while the Saudi-Indian haplotype, which is generally not seen in the African populations was recognized in the population of India in West Orissa, Qatar, Bahrain and Saudi Arabia (El-Hazmi, 1990). In a Jamaican family the Saudi-Indian haplotype was reported, however, the family was of Indian origin.

5.3.7 Beta-globin gene haplotypes in Saudis

In an attempt to investigate differences in the β-globin gene haplotype pattern in Saudi sickle cell disease produced by sequence differences which result from alteration of the recognition site for specific restriction endonucleases, we conducted studies on sickle cell disease patients, from different areas of Saudi Arabia, using Hinc II, Hind III, Ava II,

Figure 5.8: Restriction endonuclease site used to construct the β-globin gene haplotype



(Each arrow points to the approximate location of the restriction endonuclease polymorphic site which may or may not be present.. The results of 5 or more polymorphic sites are together used to construct the
β-globin gene haplotypes)

Table 5.5: Gene probes used and fragments produced upon DNA digestion with different restriction endonucleases

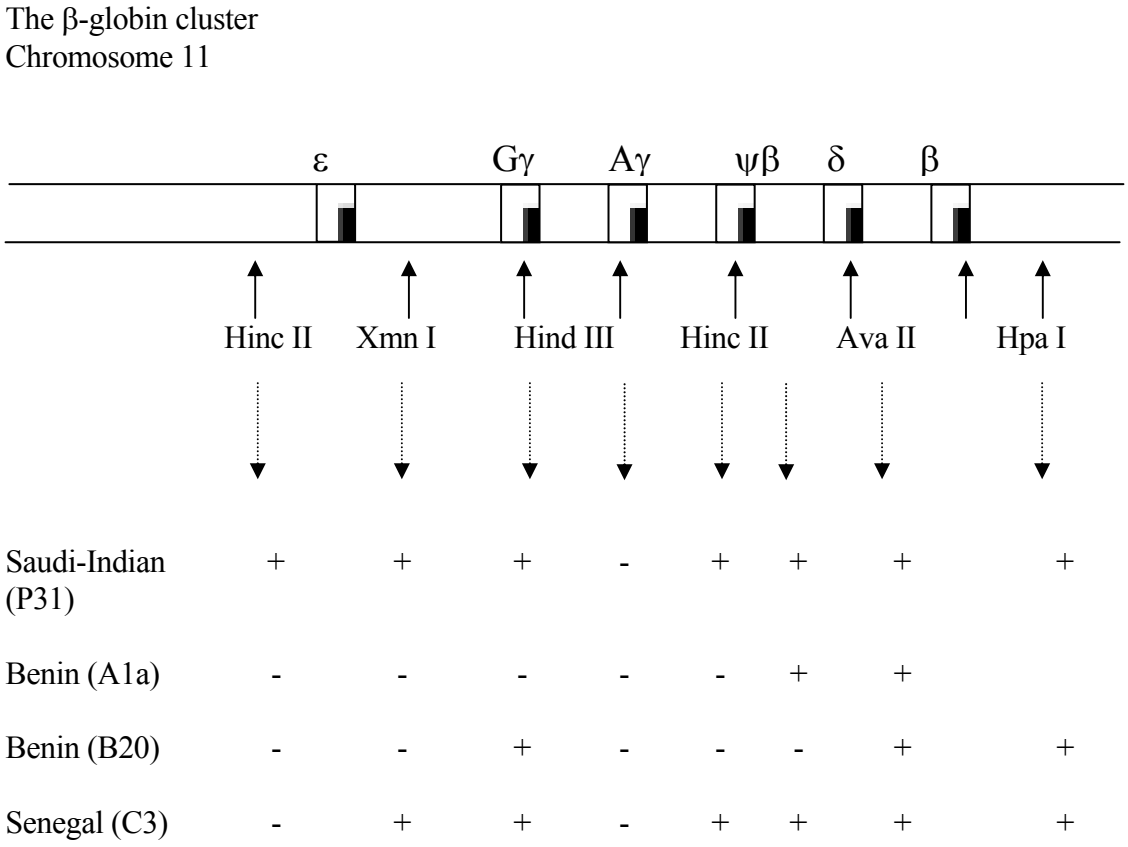
RE	32^P labeled gene probe	Polymorphic Site	Fragment size (Kb)
Hinc II	ε	(+) (-)	3.7 8.0
Xmn I	γ (CHB 4)	(+) (-)	7.0 8.1
Hind III	γ	(+) (-)	7.1, 0.7 7.8
Hind III	γ	(+) (-)	2.7, 0.7 3.4
Hinc II	ψβ	(++) (--) (-+)	3.0 7.6 6.0
Ava II	βIVS II	(+) (-)	2.0 2.2
Hpa I	5' β	(+) (-)	7.6 or 5.6 or 7.0 13.0

Table 5.6: Beta-globin gene haplotype in Saudi sickle cell disease patients in different provinces of Saudi Arabia

Eastern Province		Western Province	
Haplotype	Prevalence	Haplotype	Prevalence
++-++/++-++	38.09	----+/----+	53
++-++/-+-++	19.05	----+/-----	13
++-++/---++	9.52	----+/+----	6
++-++/----+	9.52	----+/-++-+	4
----+/--+--	4.76	---+/--+--	2
++-++/-+-++	4.76	----+/+---+	2
++-++/-+-++	4.76	----+/-+---	2
++-++/+--++	4.76	----+/--+-+	1
++-++/-----	4.76	----+/+++-+	1
		----+/++++-	1
		----+/+++--	1
		--+--/--+--	4
		-----/-----	2
		+----/+----	2
		++-++/++-++	2
		-++--/-++-+	1
		-+---/-+---	1
		+---+/+----	1
		-+---/++--+	1

No. of patients investigated in Eastern Province= 21
Western Province=100

Figure 5.9: A schematic representation of major β-globin haplotypes



Xmn I, Hpa I and determined the 5' sub haplotypes. The fragments obtained by treatment with each restriction endonuclease in presence (+) or absence (-) of the restriction site are presented in Table 5.6 and the autoradiographs showing the fragments obtained with each restriction endonuclease are shown in Figures 5.10 to 5.12. The haplotypes were constructed using the combination of results of the different restriction endonucleases and the results obtained are presented in Figure 5.13.

5.3.8 Current theories on Hb S distribution

The principal factors that have led to the distribution of Hb S in the different areas of the world are (a) the mutation taking place in the different areas and (b) the movement of affected population. The exact causes of the sickle cell mutation are unknown, however, as discussed above, there is sufficient evidence to show that the sickle cell mutation has occurred atleast at four occasions on chromosomes different from each other in terms of normal DNA structural variations (i.e. polymorphism). These restriction endonuclease polymorphic sites are located in the intervening sequences between the globin genes on chromosome 11. In Africa, the Hb S appears to have occurred on three different chromosomes carrying the Benin, Bantu and Senegal haplotype of the β-globin gene cluster (Figure 5.12). These haplotypes have been identified by restriction endonuclease studies in the Black Americans and the immigrant populations in the different parts of the world and have played an important role in the study of the movement of the Hb S gene to the Americas and the European countries. On the other hand, an independent mutation has occurred in the Indian sub-continent and Arabia as judged from the different β-globin gene haplotype identified in these populations. Whether the mutation occurred independently in

India and Arabia yet to be confirmed.

Figure 5.10: Autoradiograph of fragments produced by the presence of absence of Hinc II polymorphic site 5' to ε gene

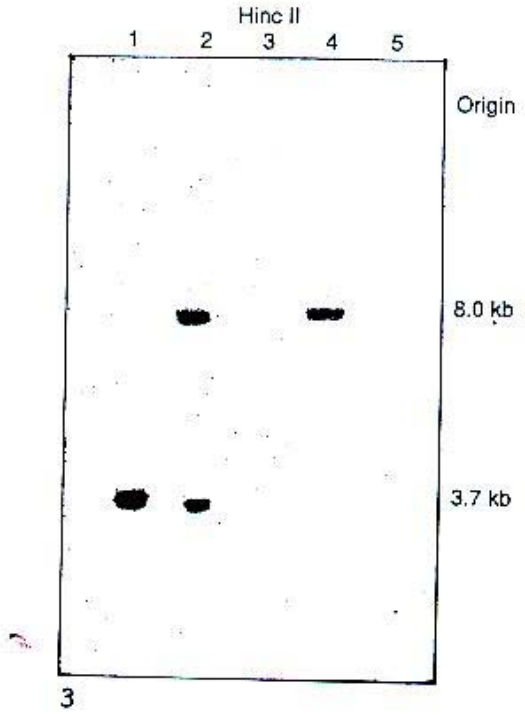


Figure 5.11: Autoradiograph of fragments produced by the presence of absence of Hinc II polymorphic site in the ψβ globin gene

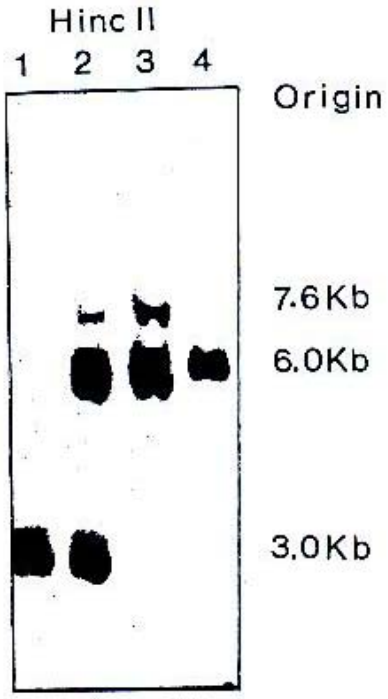

Hinc II
1 2 3 4
Origin
7.6Kb
6.0Kb
3.0Kb

Figure 5.12: Autoradiograph of fragments produced by the presence of absence of Hinc II polymorphic site 5' in Gγ and Aγ

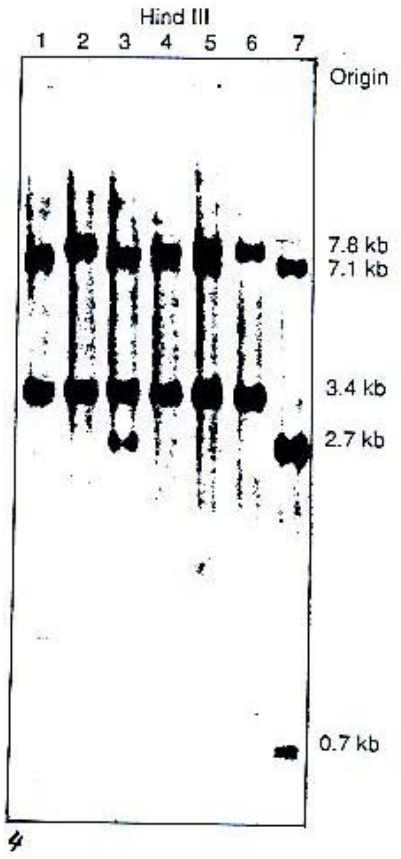


Figure 5.13: The origin and spread of β^S gene

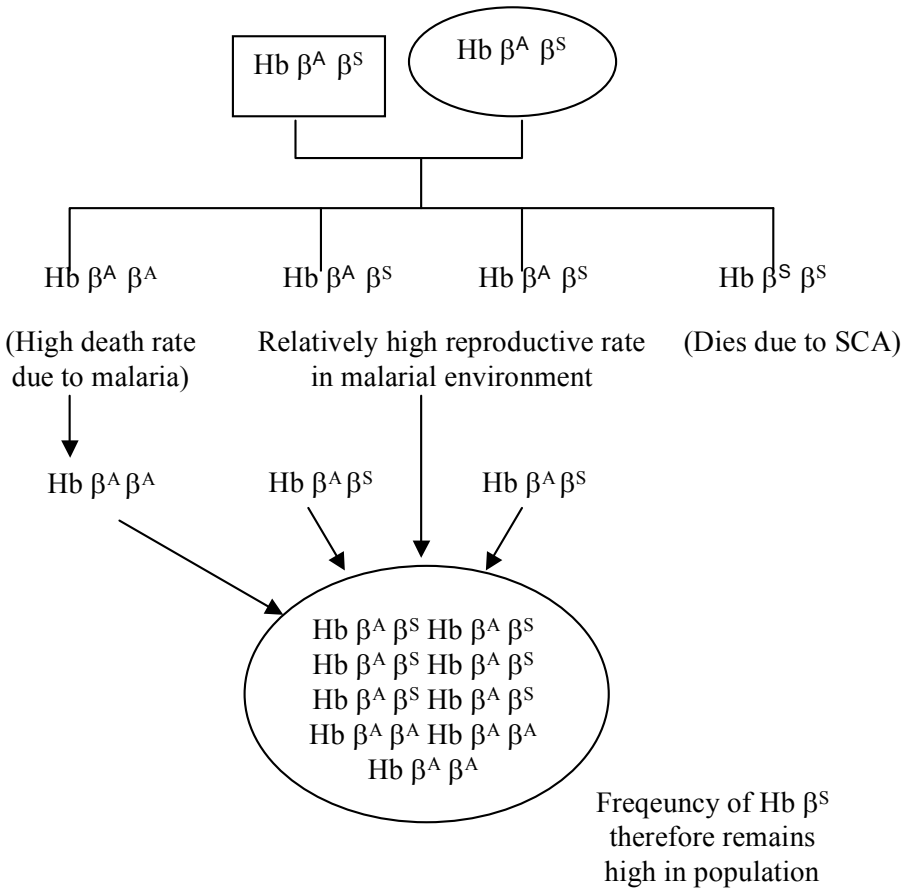
The spread of these mutations from Africa and Asia is presented in Figure 5.13. Population movement has been a major factor in the spread of the Hb S. The Hb S mutation occurred in Africa and the Hb S gene spread to Central and South Americas, the Carribeans, and the Southern United States by the population movement particularly during the slave trade days. Later the gene moved to the United Kingdom, Canada, North America, France, Netherlands, Belgium, Portugal, Germany and other European countries as a result of immigration of affected population.

The environmental factor that has played a role in the distribution and maintenance of Hb S at a high frequency is the selection by falciparum malaria. It has been shown that the sickle cell gene carrier (sickle cell trait) have an inborn resistance against the growth of malarial parasite and thus Hb S confers a protection against falciparum malaria, particularly during childhood, when malaria is most fatal. The child with sickle cell trait develops lower malarial parasitemia and is less likely to die from malaria. As the child survives into adulthood he or she breeds and passes on their abnormal genes to the offspring. Thus it can be stated that the sickle cell trait have an increased fitness over normal Hb AA and Hb SS individuals in areas with malaria endemicity and thus they have survived, resulting in the maintenance of the Hb S gene frequency (Figure 5.14).

The Saudi patients from different regions of Saudi Arabia have significantly different haplotypes. Majority of the patients from the south-western and western provinces are either homozygous or heterozygous to the Benin haplotype while the patients from the eastern province have mainly the Saudi-Indian haplotype in homozygous or heterozygous state. In the light of these findings and those of the β-globin gene Hpa I

polymorphism, it appears that there are two independent foci of occurrance of sickle cell

Figure 5.14: Balanced polymorphism: Maintenance of Hb S in an environment endemic to malaria



gene mutationin the Saudi population. One is confined largely to the Eastern province population and the other to the Western province of Saudi Arabia. Regarding the origin of sickle cell gene in Saudi population, there are several possibilities: *Firstly*, it is possible that sickle cell mutation took place in both the foci in the eastern and western provinces on chromosomes carrying different β-globin gene haplotypes and as a result of population movement these haplotypes spread within the country and the Hb S gene frequency was maintained as a result of the endemicity to malaria. From the eastern province, population movement to the Emirates, and the Indian sub-continent could have resulted in the spread of the Saudi-Indian haplotype in those regions. Similarly, the Benin haplotype could have spread to the African population. The *second* possibility is that the Benin mutation originated in the African population and spread to the western province of Saudi Arabia and was maintained by the presence of malaria endemicity. A *third* possibility is that there have been independent mutation in Saudi Arabia and the Africa or India. The haplotype in the Western Saudi Arabia being similar to the African haplotype and in the Eastern Saudi Arabia is similar to Indian haplotype, but have independent origin.

It is more appropriate to predict that the major haplotype in Saudi Arabia are the ones which arose from mutations in these regions. Additional haplotypes which occur at a low frequency are the ones which arrived and established as a result of population movement. It is well known that Arabia has throughout history been a centre of trade and attraction for Pilgrims and the movement of population has been significant. However, the main criticism that we feel against spread to Saudi Arabia, is the very low frequency of

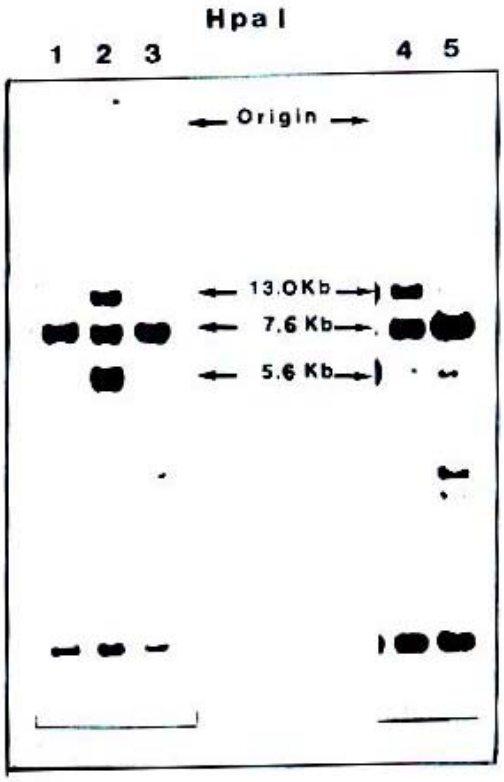
other African haplotypes i.e.Bantu and Senegal in the Saudis.

5.4 Double β-globin genes in Saudis

Five cases were identified who gave 3 bands containing β-globin gene on digestion with Hpa I (Figure 5.15). This gene arrangement may result from presence of 2 β-globin genes on one chromosome and one on the other, thus producing a triple Hpa I fragment. This could only be explained by assuming that there was duplication of β-globin gene on one chromosome as a result of unequal crossing-over during intra chromosomal translocations depicted schematically in Figure 5.16 (El-Hazmi et al, 1986).

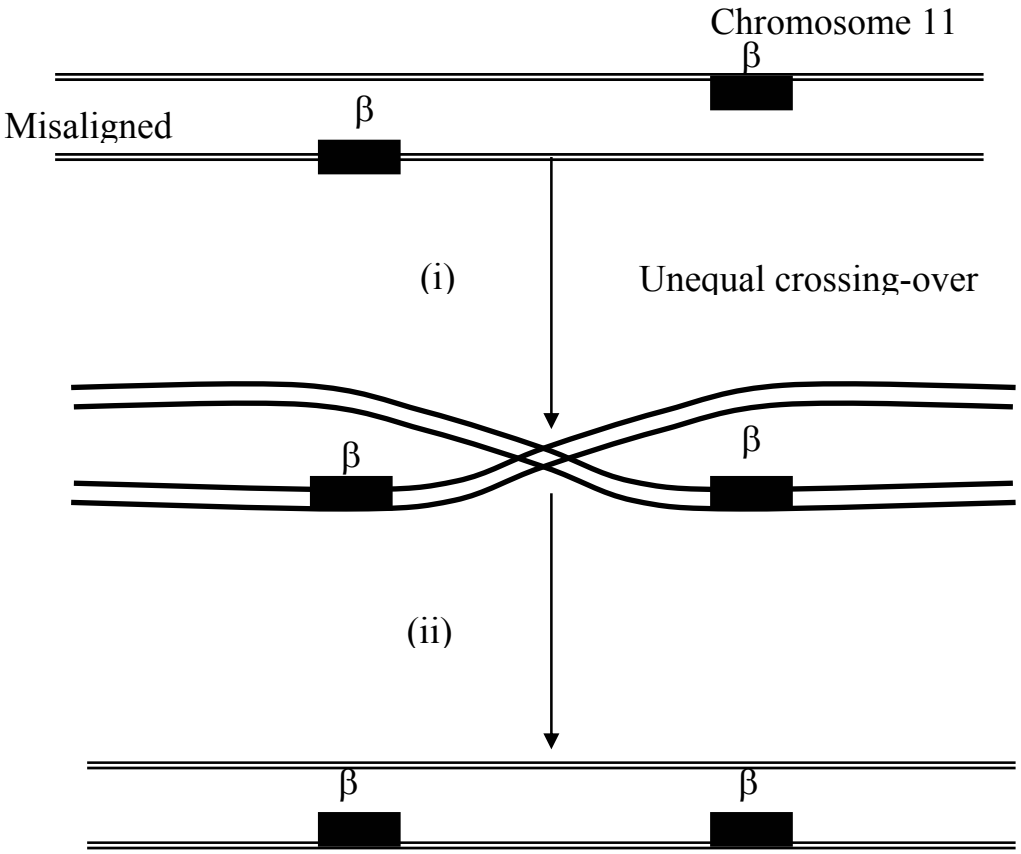
Such cases were reported for the first time in Saudi population and similar conditions could not be found in the literature.

Figure 5.15: Triple Hpa I fragment containing the β-globin gene



Sample 1, 3 and 5 - Normal with 7.6 kb Hpa fragment
Sample 4 - Heterozygote with 13.0 kb and 7.6 kb fragments
Sample 2 - Triple Hpa I fragment

Figure 5.16: Unequal crossing-over of β-globin gene, resulting in the



CHAPTER 6

SICKLE CELL HETEROZYGOTES (TRAIT) IN SAUDI ARABIA

6.1 Introduction

The Hb S heterozygous state (i.e. trait, Hb AS) is the condition resulting from the presence of Hb S mutation in only one β-globin gene on one chromosome while the other is normal. On electrophoresis both Hb A and Hb S with a minor Hb A2 band appear as separate bands and the condition can be diagnosed with certainity following electrophoresis. Further confirmation of Hb AS state may be made using the restriction endonuclease Mst II, which produces both 1.15 kb and 1.35 Kb fragments in these individuals.

Generally, the individuals with sickle cell trait are asymptomatic and do not show any significant haematological or clinical abnormalities. However, under certain conditions, such as low oxygen partial pressure, episodes of crises may be precipitated.

6.2. Level of Hb S in Hb S heterozygotes

The level of Hb S in the sickle cell trait cases shows a wide range between 20-45 % (Itano, 1953). It is generally always lower than Hb A level and the mechanisms involved is lowering the Hb S production compared to Hb A production are suggested to be as follows:

i) reduced affinity of available α-chains for β^S-chains compared to the affinity for β^A-chains (Abraham and Huismann, 1977);

ii) lower stability and, therefore, increased rate of turnover of β^S-chains compared to β^A chains (DeSimone et al, 1974);

iii) different rates of synthesis of Hb S compared to Hb A (Heywood et al, 1964; Bank et al, 1970), and

iv) increased binding of Hb S to cell stroma (DeSimone et al, 1977) and, therefore, lower level in the red cells.

The distribution of Hb S level in heterozygotes in most populations is shown to be negatively shewed and the mean is reported between 34-38 percent. The Hb S level varies in different individuals and is determined by genetic and environmental factors. The environmental factors which influence the Hb S level include associated iron deficiency or megaloblastic anaemia, while the genetic factors with a major influence on Hb S level are associated α- or β-thalassaemia states.

The proportion of Hb S in heterozygotes is considered as an indicator for determining the presence or absence of α-thalassaemia (Brittenham et al, 1979). It has been shown that lower values of Hb S are indicative of co-existing α-thalassaemia and furthermore, the quantity of Hb S in heterozygotes is inversely related to the number of α -Thalassaemia genes present (Wong et al, 1981).

Table 6.1 summarizes the mean Hb S level reported in Hb S heterozygotes in different population with normal α-globin genes (αα/αα), one α-gene deletion (-α/αα) and two α gene deletions (-α/-α).

The heterozygous distribution of the Hb S level has been explained by a genetic model, according to which the active α-gene loci can modify the net synthesis of Hb S. This model indicates that the presence of α-thalassaemia is a cause for the decreased amount of available α chains. This further leads to a decreased net synthesis of Hb S because of the lower affinity of the α-globin chains for the β^S chains, compared to that for the β^A chains. Both biosynthetic and DNA studies have confirmed this model

Table 6.1: The mean value of Hb S in Hb S heterozygotes in different populations

Population	Hb S (%) mean value in association with:			Reference
	αα/αα	-α/αα	-α/-α	
California	40.0	31.6	24.8	Embury & Dozy, 1979
India	36.8	31.6	24.8	- Brittenham et al, 1977 - Abraham & Huisman, 1977
Georgia	41.2	35.5	28.1	Huisman, 1977
Ontario	40.4	35.1	28.6	Wong et al, 1981
Jamaica	36.0	31.0	24.0	Brittenham, 1977
Saudis	40.0	31.5	23.0	El-Hazmi, 1986

(Felice et al 1978; Brittenham et al, 1980). Applying this model, the genotypes in Hb S heterozygotes with low, medium and high Hb S level are -α/-α, β^A/β^S; -α/αα, β^A/β^S and αα/αα, β^A/β^S, respectively.

Presence of β^+ thalassaemia results in increased Hb S level, while presence of β°-thalassaemia produces complete absence of Hb A and hence double heterozygous state, termed as Hb S/β°-thalassaemia, which is electrophoretically, haematologically and clinically similar to homozygous sickle cell has only no Hb A.

6.3 Hb S level in Saudi Hb S heterozygotes

After excluding Hb S/β^+ and Hb S/β°-thalassaemia cases, the Hb S level in the Saudi heterozygotes shows a trimodel distribution as presented in the frequency distribution histogram (Figure 6.1). The mean of Hb S in the three major peaks is 23, 31.5 and 40 percent while the range in the three peaks is 18-28; 28-35, and 35-45 percent, respectively. The distribution of Hb S in individuals with different α gene deletions diagnosed using Bam HI are presented in Figure 6.2.

Another interesting finding in the Saudis is the prevalence of α-thalassaemia in the Hb S heterozygotes in the different regions of the country (Figure 6.3). The highest prevalence of α-thalassaemia in the Hb S heterozygotes is in the eastern province of Saudi Arabia, while the lowest prevalence is in the north-western provinces. When grouped on the basis of their origin, slight differences are encountered in the mean and range of Hb S level in the Hb S heterozygotes, though the differences are not statistically significant.

The high rate of co-existence of these two genetic abnormalities cannot be explained on the basis of any genetic linkage as the α globin genes are located on

Figure 6.1:Frequency distribution histogram of Hb S level in Saudi Hb S heterozygotes

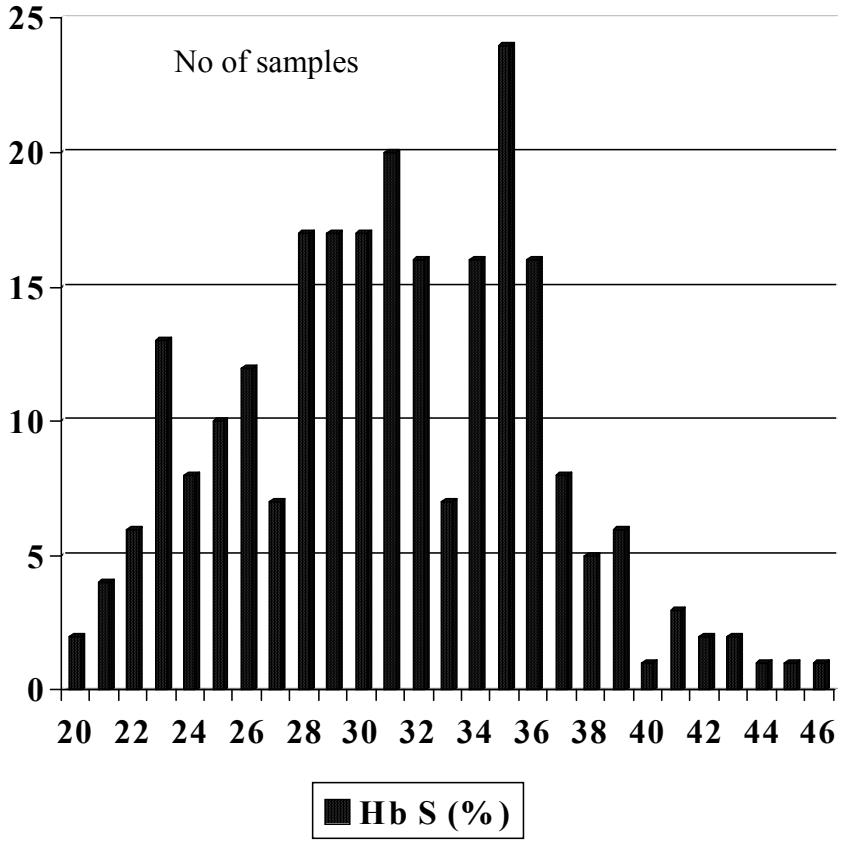
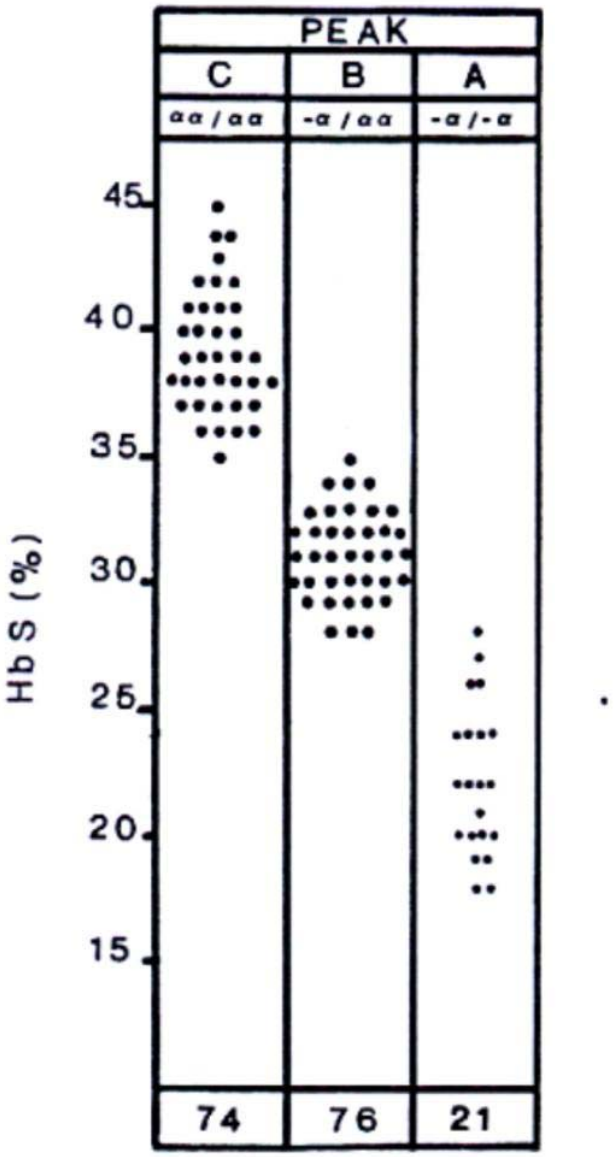


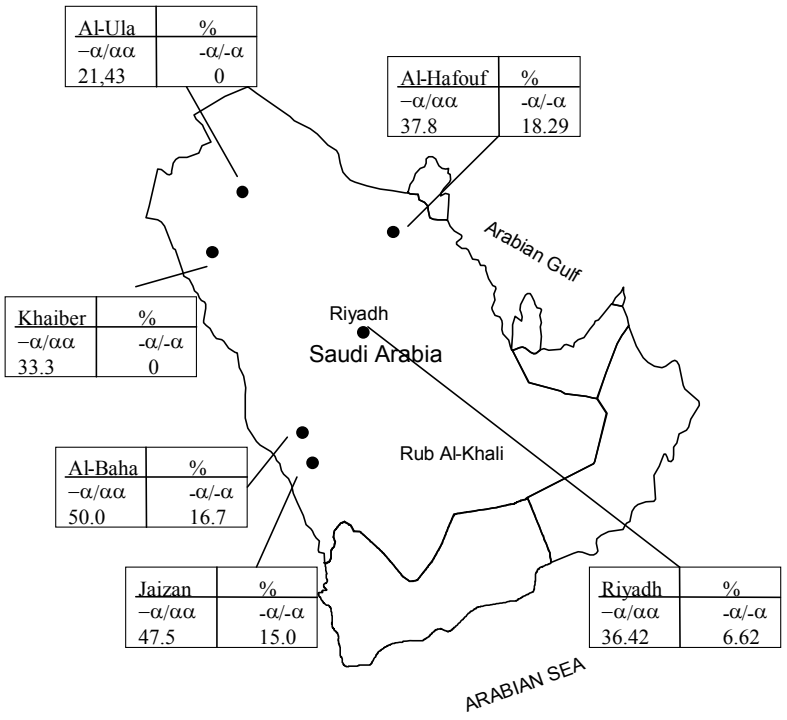
Figure 6.2: Hb S level in heterozygotes with difference number of α-globin genes*



*Diagnosed using Bam HI. The number in the lower boxes is the number of Hb AS individuals investigated

Figure 6.3: Frequency of alpha-thalassaemia in Hb AS individuals
From different regions of Saudi Arabia

-



chromosome 16 while the β-globin genes are located on chromosome 11. However, in Saudi Arabia, both Hb S and α-thalassaemia genes occur at a high frequency in the same area, and this high prevalence of co-occurrence of sickle cell and α-thalassaemia genes may be a resultant of the high rate of consanguinity, and marriages within the same tribes and other forms of intermarriage customs which are prevalent in the population of Saudi Arabia. Furthermore, the presence of Hb S or α-thalassaemia in the carrier state plays a protective role against the harmful environmental factors such as malaria, it is possible that the co-existance of both Hb S and α-thalassaemia have a further beneficial effect on the survival of the double heterozygous individuals in presence of malaria.

6.4. Clinical abnormalities in Hb S heterozygotes

Clinically sickle cell heterozygote state is considered to be benign, however, there is significant evidence that certain abnormalities do occur in the carrier state. This is specially true under conditions resulting in hypoxia such as that encountered at high altitude. Generally, the presence and severity of clinical manifestations in the Hb S heterozygotes depends on:

i) the level of Hb S in the red cells;

ii) the presence of other red cell genetic abnormalities e.g. the thalassaemias, G-6-PD deficiency;

iii) the presence of nutritional deficiencies e.g. folate or iron deficiency;

iv) presence of both (ii) and (iii) together;

v) the level of other haemoglobins i.e. Hb F.

The W.H.O. Technical Report (1966) states that the morbidity and mortality in individuals heterozygous to Hb S is no different from the normal individuals. This statement is based on several studies on populations that have shown the survival is not impaired in individuals who are Hb S heterozygotes. However, going through the literature one comes across several case reports of Hb S heterozygotes with clinical complications, though from these case reports it is not possible to obtain the prevalence of the clinical abnormality.

The pathophysiological mechanisms which may result in complications in the patients with sickle cell trait are presented in Figure 6.4. The pathogenicity depends on the degree of anoxia, which is determined by the oxygen tension, gas transfer to the lungs and the local circulatory conditions.

The spleen and the kidney seem to be most affected in the Hb S heterozygotes. In the splenic vascular bed a sluggish circulation exists and under conditions of hypoxia such as that caused by high altitude, vaso-occlusive episodes may occur. Similarly in the renal medulla, condition of low oxygen tension, altered pH and tonocity also favour sickling, thus resulting in haematuria, renal papillary necrosis and bacteriuria.

In other organs the reports of pathogenicity are less frequent. However, as explained by Konotey Ahulu (1969a,b) the sickle cell heterozygotes differ from the sickle cell homozygotes simply in the amount of Hb S (quantitative difference) and under appropriate alterations in the internal environment, the Hb S heterozygotes may suffer from the same complications of the Hb S homozygotes.

In the following section, the major and rare complications (Table 6.2) proven to

occur in the Hb S heterozygotes and complications reported in a few case reports but not

Table 6.2: Complication in HbS Heterozygotes

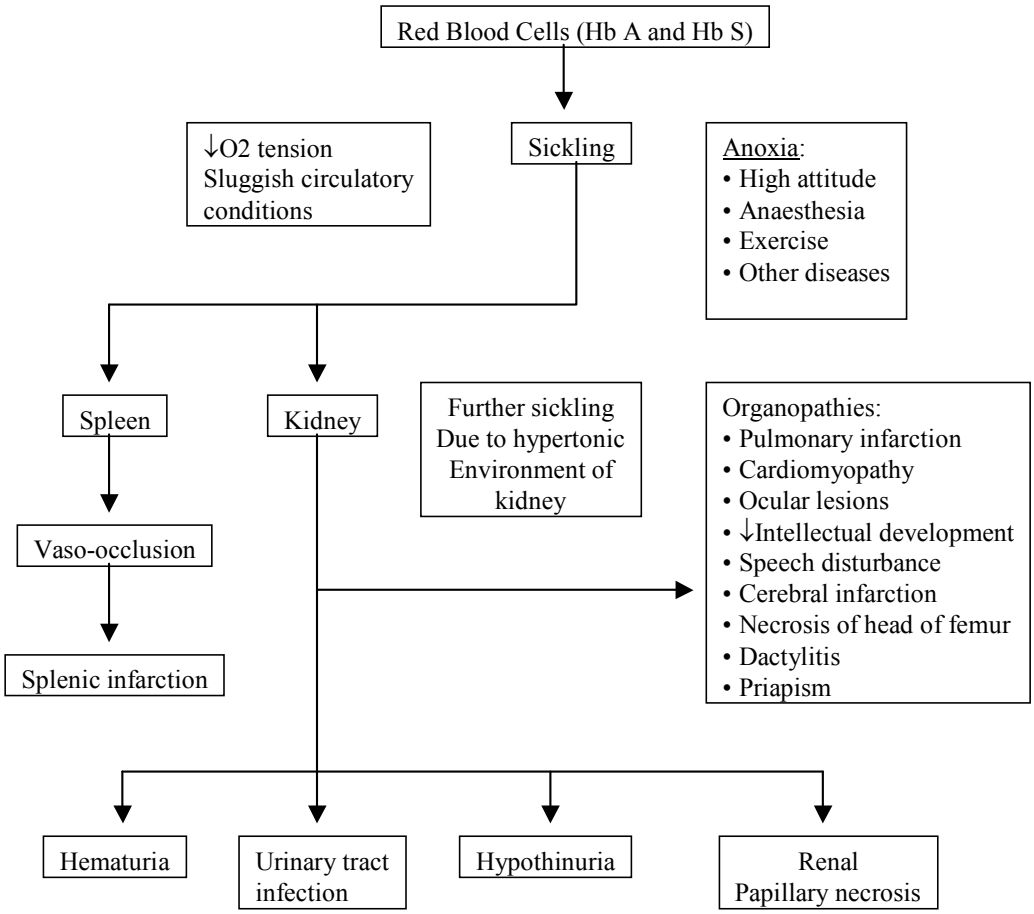
Proven Complications		Not proven Complications
Common	Rare	
Hematuria	Pulmonary & CVS complications	- Shortened survival - Enhanced morbidity
Hyposthenuria	Ocular	- Frequent hospitalization - Hepatic infarction
Renal Papillary Necrosis	Neurological	- Retarded growth - Hypotonia of urinary bladder
Bacteruria in non-pregnant women	Anaestheisa	- Cholelithiasis
Splenic Syndrome		- Leg ulcers - Retroperitoneal fibrosis
Bacteria and pyelonephritis in pregnant women		- Panhypopituitarism - Dupuytren's contracture

Table 6.3: Clinical complications observed in Saudi Hb S heterozygotes

Complications	No. of cases
1. Renal	
• Haematuria	1
• Hyposthenuria	0
• Urinary tract infections	2
2. Spleen	
• Infarction	Nil
• Splenomegaly	14*
• Splenectomy	1
3. Bone & joint pain	10*
4. Hepatomegaly	7*
5. Hand-foot syndrome	3*
6. Crises:	
• Vasoosslusive	10*
• Infective	Nil
• Haemolytic	1
• Aplastic	Nil
7. Blood transfusions	11*
8. Leg ulcers	Nil
9. Priapsim	Nil

Total number of Hb AS cases = 489
* Some patients had associated thalassaemia or other disorders

Figure 6.4: Pathophysiology of complications in Hb S heterozygotes



proven to be related to Hb S heterozygous state are outlined. Table 6.3 lists the complications encountered in the Saudi Hb S heterozygotes.

1. Renal complications in Hb S Heterozygotes

In the kidney the conditions are generally hypoxic, hypertonic and acidic and thus sickling is frequently induced in Hb S heterozygotes. The frequent sickling under normal conditions may lead to the disruption of the *vasa rectae* system in the renal in Hb AS patients medulla and thus result in renal tubular function disturbances. These include haematuria, renal papillary necrosis and increased prevalence of urinary infection.

(a) Haematuria: The exact frequency of haematuria in the Hb S heterozygotes is not known. However, in several studies haematuria has been reported in Hb S heterozygotes. In a 10 years follow-up study in Jamaica, 1% of 119 Hb S heterozygotes were reported to have developed haematuria (Ashcroft and Desai, 1976). In pregnant Hb S heterozygote female the frequency of haematuria (16.5%) was more than in the normal females (6%) (Tuck et al, 1983). During our studies only one Saudi Hb S heterozygote female was found to have haematuria, but this could have been due to some other cause.

(b) Hyposthenuria: A defect in the urine concentrating mechanism due to persistent vascular occlusive destruction of the *vasa recta* which supplies the long loops of Henle, is reported to occur in a high proportion of Hb S heterozygotes. In the Saudi population we did not encounter hyposthenuria in any case.

(c) Infections: Urinary tract infections and their recurrence is more common in Hb S heterozygotes females, particularly during pregnancy. A higher prevalence of pyelonephritis is also reported during pregnancy and increases with increase in Hb S level.

Among the male population no significant differences are reported in the prevalence of bacterial infections in Hb S heterozygotes or normal controls. Two Saudi Hb S heterozygote females complained of repeated urinary tract infections.

2. Splenic Infarction

The spleen seems to provide an ideal environmental for sickling and thus splenic infarction have been reported particularly under conditions of low oxygen tension, as that encountered at high altitude or during flying in an unpressurized aircraft. This splenic infarction has also been reported in an athlete following vigorous exercise and during prolonged breath holding, diving or under water swimming. However, there are several controversial reports regarding the risk of diving accidents in the Hb S heterozygotes. We did not encounter any Saudi Hb S heterozygote who suffered from splenic infarction. Though splenomegaly was encountered in 14 patients, ten of whom had suffered vaso-occlusive crises. In one Hb AS case splenectomy had to be performed.

3. Rare complications

Other rare abnormalities that have been confirmed to occur in Hb S heterozygotes include:

(a) Ocular Abnormalities: The exact frequency of ocular abnormalities in Hb S heterozygotes compared to normal controls has not yet been established. However, in a few reports a wide spectrum of ocular abnormalities have been reported in Hb S heterozygotes. These abnormalities lead to proliferative retinopathy and include glaucoma, acute chorio-retinal infarction, vitreous haemorrhage, retinal haemorrhage and exudates, and retinitis proliferans. What was the basic contribution of Hb S heterozygosity in the

development of ocular complications is to be established. We did not see this complication in the Saudi HbS heterozygotes.

(b) Neurological Complications: A few case reports present data on neurological complications in Hb S heterozygotes and the exact prevalence of these abnormalities is not known. In addition, these case reports are not entirely conclusive since the diagnosis of the Hb S heterozygote state was not made definitely. However, the neurological abnormalities reported in Nigerian patients include localized weakness, sensory loss, speech disturbances, migraine, loss of consciousness and equilibrium, ophthalmophegia and low intellectual measures. However, other reports have failed to find any abnormality in Hb S heterozygotes. Multiple cerebral infarction were confirmed in a study on Hb S heterozygotes cases. We did not encounter any Saudi Hb S heterozygous patient with any specific neurological complications which could be related to the Hb S heterozygous state.

(c) Cardio-pulmonary complications: In a study on Hb S heterozygotes and a large control group the prevalence of pulmonary embolism was significantly more in the Hb heterozygotes. In other studies, well documented cases of pulmonary infarction have been reported. In one of these cases the infarction was precipitated by high altitude in a patient who had a long history of progressive exertional intolerance (Nussbaum and Rice, 1984).

Deleterious effects of Hb S heterozygosity has been reported in heart disease patients. Haemolysis and splenic sequestration was reported during an episode of cardiac decompensation in an Hb AS patient with an arteriosclerotic disease (Sears, 1979). In other studies no evidence of increased myocardial infarction was demonstrated between Hb S

heterozygote and normal controls. The Saudi Hb S heterozygotes did not show any specific cardiac related problems.

(d) Bone and Joint Lesions: A few case reports have described bone and joint lesions including aseptic necrosis of the femoral head, widening of medullary cavity and Salmonella spondylitis (Sears, 1978). Dactylitis was reported in Hb S heterozygous child. However, in other studies no specific increase in the incidence of bone or joint involvement could be demonstrated between patients and control group (Sears, 1978). Unusual bone and joint pain were encountered in 10 of the patients followed during our study. Three of the Hb S heterozygous with a severe form of the disease suffered from hand-foot syndrome and was admitted at King Khalid University hospital. They also had hepatomegaly, splenomegaly, and one had to have splenectomy pains in bones and joints and had episodes of vaso occlusive crises.

(e) Leg Ulcers: A few studies have reported a higher frequency of leg ulcers in Hb S heterozygotes compared to the control group. No case of leg ulcers was encountered in the Saudi Hb S heterozygotes.

(f) Priapism: A few cases of Hb S heterozygosity with priapism have been reported. These are explained on the basis of local condition of stasis and hypoxia in the vasculature of the corpora cavernosa. No Saudi Hb S heterozygote showed this abnormality.

(g) Effect of exertion in Hb S heterozygotes

Controversial reports are found in the literature concerning the effect of excessive exertion in Hb S heterozygotes. In a few studies, no difference could be demonstrated compared to

the control group. A few case studies reported of renal failure, disseminated intravascular coagulation and sudden death on strenuous exertion in Hb S heterozygotes. In a recent study, sudden unexplained death was found to occur more in Hb S heterozygotes at the age of 20-30 years compared to a control group of the same age. We did not follow the Hb S heterozygotes though any strenuous exertion, though following normal exertion, no specific abnormality was encountered in the Saudi Hb S heterozygotes.

(h) Effect of Anaesthesia

The risk of sickling in Hb S heterozygotes, following anaesthesia is small if oxygen supply is adequate. However, complications (sickle cell crises, splenic infarction, cerebral infarction and superior longitudinal thrombosis have necessitated the careful monitoring of oxygen during surgery of Hb S heterozygotes (Sears, 1978).

(i) Pregnancy and Hb S heterozygosity

There is evidence that certain abnormalities occur at a higher frequency in sickle cell heterozygotes compared to normal individuals particularly during pregnancy. These include hyposthenuria, renal hematuria, bacteriuria and pyelonephritis during pregnancy and splenic infarction and anaemia with high altitude hypoxia (Eisenstein et al, 1956; Sears, 1978).

Several studies have been conducted on pregnant Hb S heterozygous females to access the major abnormalities and no differences were found between the patients and the control groups in the frequecy of premature labour, peripartum haemorrhage, maternal mortality, fetal loss and still birth and frequency of Caesorean section. In one study, a signifycantly lower birth weight was reported in babies of mother with Hb S heterozygosity and it has

been explained by suggesting that reduced oxygen delivery to the fetus may result in reduced birth weight. In another study the mean birth weight was found to be lower in the Hb S heterozygous females and the femur length was significantly lower when measured at each gestational period, compared to females with normal haemoglobin. The frequency of abortions has been studied in several investigations and apart from one report stating a higher frequency of abortions in the Hb S heterozygotes, all other reports fail to show any difference in the frequency of abortion in the patients and the control group. Similarly the number of pregnancy and the prevalence of subfertility were the same in the two groups (Tuck et al, 1983).

We investigated 21 sickle cell heterozygous (Hb AS) females in whom pregnancies were followed for a period of 5 years and the `control group' included 26 pregnancies in 26 normal Hb AA females. The patients and control groups were matched for variables including maternal age, parity, body mass index, previous abortion(s), previous low birth weight (LBW) babies, previous preterm deliveries, previous perinatal deaths, rate of labour induction, gestational age at delivery and haemoglobin level during late pregnancy. Physical measurements were carried out using ultrasonic measurement, and the patients and controls were assessed for Biparietal Dimension (BPD), femur length and abdominal circumference. The anthropometic measurements included the measurement of birth weight, head circumference, crown-heel length and placental weight. Apgar score was obtained for each patient and control. During the pregnancy, the patient and controls were regularly followed every four weeks from the 14th week onwards.

Our study did not show any difference in the frequency of abortions, previous low

birth weight babies, previous preterm deliveries, previous perinatal death, parity, femur length, abdominal circumference, birth weight and crown-heel length in the two groups we investigated at different stages of gestation (Khashogi et al, 1992).

However, a significant difference was observed at 34th week, in the BPD, the head circumference and placental weight. This could be a consequence of individual variations and not a consequence of sickle cell heterozygocity.

The low birth weight babies in the two groups did not differ in the patient (15%) and control (8%) group (P>03), similarly intrauterine growth retardation was 4% in both the groups.

6.5. Haematological and biochemical parameters in Saudi Hb S heterozygotes.

The value of haematological parameters in Hb S heterozygotes with no other associated abnormality are generally within the normal range under normal conditions. The red cell survival is normal and, therefore, no significant abnormality is encountered (El-Hazmi et al, 1987).

We investigated 242 Hb S heterozygotes from the eastern province and 247 heteroyzgotes from the western province of Saudi Arabia. The haematological parameters in the adult males and females and children group compared to the Hb S heterozygotes from the western province are presented in Table 6.4.

As seen for the normal Hb AA individuals, variations were encountered in the levels of the various haematological parameters in the Hb AS group. The total haemoglobin level and Hb F level in the Hb AS adult males, females and children in the different regions are presented in Tables 6.5 and 6.6. Differences were encountered in the

value of the various haematological compared to the normal group, though were not statistically significant. Variation in Hb F level were encountered and ranged from 0.2% to 3.5%.

Table 6.4: The haematological parameters in Saudi Hb S heterozygotes from the western and eastern provinces

Parameter	Hb AS (EP)			Hb AS (WP)		
	Male	Female	Children	Male	Female	Children
No.	75	98	69	69	93	85
Age (yrs)	34.2 ±14.8	31.4±12.0	8.8 ± 3.9	33.6 ± 14.9	33.6 ± 14.9	9.04± 3.2
Hb (g/dl)	13.4 ± 1.9	11.9 ± 1.5	11.7± 1.3	14.0 ± 1.6	12.0 ± 1.9	11.7 ± 1.6
PCV (l/l)	0.43± 0.05	0.38±0.04	0.37±0.04	0.41±0.035	0.35±0.05	0.34±0.05
MCV (fl)	81.0 ± 5.0	76.0 ± 9.3	78.0 ± 7.7	79.7 ± 6.0	79.1 ± 7.6	78.8 ± 6.3
MCH (pg)	25.0 ± 3.9	24.0 ± 3.3	26.0 ± 2.9	28.4 ± 2.1	26.9 ± 2.7	27.3 ± 3.7
MCHC (g/dl)	31.6 ± 3.0	31.1 ± 1.3	31.8 ± 1.7	34.0 ± 2.0	33.1 ± 2.9	33.0 ± 1.9
Hb A_2 (%)	3.0 ± 0.5	3.0 ± 0.5	3.1 ± 2.0	2.6 ± 0.27	2.5 ± 0.27	2.5 ± 0.3
Hb F (%)	0.9 ± 0.5	1.1 ± 0.6	0.8 ± 0.4	1.9 ± 0.4	1.9 ± 0.4	2.6 ± 0.5
WBC ($x10^9$/l)	5.6 ± 1.6	5.9 ± 2.4	8.0 ± 5.6	7.5 ± 3.9	7.5 ± 3.9	8.2 ± 4.4

Table 6.5: Total haemoglobin level in Hb AS individuals in different regions of Saudi Arabia compared to the normal (Hb AA) individuals from the same region

Region	Hb AS group			Hb AA group		
	Male	Female	Children	Male	Female	Children
Qassim	14.6 ± 1.0	-	-	14.4 ± 1.32	12.3 ± 1.49	13.3 ± 1.53
Sulayel	15.19 ± 0.98	12.78 ± 1.34	9.71 ± 4.69	15.1 ± 1.37	13.5 ± 1.63	13.4 ± 1.66
Al-Hafouf	15.68 ± 1.19	13.1 ± 1.16	13.1 ± 2.05	15.3 ± 1.8	13.2 ± 1.5	13.3 ± 2.0
Al-Qateef	11.86 ± 1.47	11.86 ± 1.47	11.66 ± 1.29	12.46 ± 1.3	11.71 ± 1.28	11.43 ± 1.31
Khaiber	15.62 ± 2.15	13.18 ± 0.67	13.16 ± 0.67	15.9 ± 1.83	14.4 ± 2.14	14.7 ± 2.1
Jaizan	13.59 ± 2.54	11.67 ± 2.23	11.75 ± 1.55	13.5 ± 2.42	11.6 ± 1.87	10.6 ± 2.2
Najran	14.73 ± 1.26	12.94 ± 1.45	13.93 ± 9.64	16.8 ± 1.56	14.5 ± 1.19	14.2 ± 1.29
Al-Baha	13.98 ± 2.31	13.31 ± 1.24	10.38 ± 1.95	15.64 ± 1.8	13.68 ± 1.8	13.23± 2.01
Bisha	16.16 ± 4.71	15.4 ± 2.9	15.42 ± 2.88	16.13 ± 2.68	14.46 ± 2.52	14.13± 2.76
Majarda	14.66 ± 1.95	11.84 ± 2.22	12.56 ± 1.77	-	-	-
Yanbu	15.22 ± 0.78	11.55 ± 0.37	11.38 ± 0.73	14.5 ± 1.43	12.56 ± 1.51	12.78± 1.46

Table 6.6: Total haemoglobin F level in Hb AS individuals in different regions of Saudi Arabia compared to the normal (Hb AA) individuals from the same region

Region	Hb AS group			Hb AA group		
	Male	Female	Children	Male	Female	Children
Sulayel	1.47 ± 0.58	1.97 ± 0.67	6.38 ± 10.61	1.0 ± 0.56	1.0 ± 0.05	2.3 ± 1.08
Al-Hafouf	0.89 ± 0.48	1.12 ± 0.63	0.84 ± 0.41	1.0 ± 0.5	1.0 ± 0.3	1.23 ± 0.2
Al-Qateef	1.34 ± 0.88	1.34 ± 0.88	1.75 ± 1.49	1.0 ± 0.5	1.93 ± 3.19	2.90 ± 5.01
Khaiber	0.67 ± 0.29	0.63 ± 0.28	0.75 ± 0.51	1.0 ± 0.24	1.0 ± 0.23	1.0 ± 0.19
Jaizan	1.45 ± 0.85	1.44 ± 0.60	1.98 ± 0.92	1.3 ± 0.67	1.3 ± 0.76	1.5 ± 1.2
Najran	0.87 ± 0.586	1.30 ± 0.82	2.87 ± 3.77	0.5 ± 0.27	0.4 ± 0.26	0.5 ± 0.2
Al-Baha	2.02 ± 2.04	2.47 ± 0.67	3.09 ± 1.72	1.12 ± 0.98	1.27 ± 1.01	1.55 ± 1.23
Majarda	0.82 ± 0.56	1.95 ± 4.45	1.33 ± 1.18	1.0 ± 0.6	1.0 ± 0.6	1.0 ± 0.6

Table 6.7: The biochemical parameter values in Hb S heterozygotes from the Western and Eastern Provinces of Saudi Arabia

Parameter	Hb AS (EP)			Hb AS (WP)		
	Male	Female	Children	Male	Female	Children
Liver Function Test						
T. Bil (ىmol/l)	6.1 ± 5.0	4.1 ± 3.2	7.8 ±10.8	6.2 ± 3.7	7.5 ± 8.1	8.6 ± 10.1
D. Bil (ىmol/l)	1.6 ± 3.3	0.5 ± 1.2	1.0 ± 1.4	1.0 ± 0.8	1.2 ± 0.5	1.0 ± 0.8
TAG (mmol/l)	2.2 ± 0.7	1.1 ± 0.6	1.0 ± 0.6	1.2 ± 0.9	0.94 ± 0.6	0.54±0.32
Chol. (mmol/l)	3.8 ± 2.1	3.9 ± 2.0	3.9 ± 2.0	4.2 ± 0.9	4.3 ± 1.2	4.6 ± 0.8
ALP (U/l)	101.8±38.0	63.5 ±19.2	213 ± 77	111.1±75	113 ± 14.0	190± 92
SGOT (U/l)	42.3±17.7	25.3 ±16.7	35.4±18.5	11.2±12.2	11.1 ± 10.5	6.3 ± 4.8
SGPT (U/l)	15.4±9.7	7.5 ± 6.9	15.3±16.8	32.8±12.0	28.1 ± 14.5	39.4 ± 9.7
T. Protein (g/l)	69.1±12.3	71.2 ±9.9	74.2±9.6	76.4± 6.4	73.9 ± 9.1	77.5± 6.1
Albumin (g/l)	43.6±5.4	43.7 ± 5.6	44.8±3.2	39.9 ± 4.6	38.4 ± 5.5	40.9 ± 3.1
Renal Function Test						
Creatinine (mmol/l)	72 ± 17.6	53.2 ±11.3	44.5 ±20.8	89.8±21.7	78.7 ±17.3	72.0±14.3
Urea (mmol/l)	4.0 ± 2.5	4.0 ± 1.8	4.0 ± 1.8	5.8 ± 1.4	4.9 ± 1.9	4.6 ± 1.9
Bone Function Test						
Calcium (mmol/l)	1.8 ± 0.6	22.0 ± 0.2	2.2 ± 0.2	2.4 ± 0.3	2.4 ± 0.3	2.4 ± 0.2
Phosphate (mmol/l)	1.2 ± 0.6	1.1 ± 0.3	1.3 ± 0.3	1.18 ± 0.3	1.06 ± 0.2	1.4 ± 0.3

Table 6.7 contd.....

Parameter	Hb AS (EP)			Hb AS (WP)		
	Male	Female	Children	Male	Female	Children
Electrolytes						
Sodium (mmol/l)	137.5±11.5	140.9±4.0	138±8.3	140.7±3.9	140.9±7.9	141±5.3
Potassium (mmol/l)	6.9 ± 2.1	5.5 ± 1.7	5.1± 0.8	4.2 ± 0.8	4.2 ± 0.9	4.1 ± 0.8
Chloride (mmol/l)	99.3 ± 2.0	100±2.5	99.1 ±4.7	106±7.0	105±7.9	106±3.2
Miscellaneous						
Uric Acid (mmol/l)	0.273±0.11	0.241±0.09	0.231±0.07	0.329±0.13	0.274±0.125	0.203±0.96
Glucose (mmol/l)	4.4 ± 4.0	3.8 ± 3.7	3.3 ± 1.4	5.0 ± 1.2	5.6 ± 1.1	4.2 ± 1.1
LDH	ND	ND	ND	174±87	183.8±72	222.2±74
Fe	30 ± 11.0	33.9 ± 11.8	ND	12.6±2.0	12.6 ± 2.8	12.9 ± 2.3
TIBC	ND	ND	ND	58.1±10.4	57.5 ± 10.7	57.0±12.1

Table 6.8: Haematological parameters in Hb AS cases with or without α-thalassaemia

Haematological Parameters	Group A (n = 21) Hb S <28% (mean ± SD)	Group B (n = 76) Hb S 28-35% (mean ± SD)	Group C (n = 74) Hb S 35-45% (mean ± SD)	Statistical significant (P<0.05)
Hb (g/dl)	13.8 ± 1.2	14.5 ± 1.3	14.5 ± 1.2	A < B = C
RBC ($x10^{12}$/l)	5.7 ± 0.9	5.0 ± 0.6	5.0 ± 0.5	A < B = C
PCV (l/l)	0.40 ± 0.03	0.42 ± 0.03	0.41 ± 0.03	A = B = C
MCV (fl)	69.0 ± 5.0	77.2 ± 5.0	80.2 ± 4.7	A < B < C
MCH (pg)	24.0 ± 2.0	28.1 ± 1.9	29.0 ± 2.0	A < B = C
MCHC (g/dl)	34.0 ± 1.6	34.5 ± 1.3	35.0 ± 1.3	A = B = C
Haemoglobin Type				
Hb A_2 (%)	2.7 ± 0.3	2.7 ± 0.3	2.8 ± 0.3	A = B = C
Hb F (%)	0.70 ± 0.40	0.45 ± 0.20	0.40 ± 0.30	A < B = C
Hb S (%)	23.0 ± 2.5	31.5 ± 2.5	40.0 ± 2.5	A < B < C

The biochemical parameters i.e. the liver function tests, renal function tests, bone function and electrolytes were analyzed in the Hb S heterozygotes from the eastern and western provinces and the mean and range are presented in Table 6.7. No statistically significant differences were encountered in the results of the two groups.

6.6. Haematological and biochemical findings in Saudi Hb S heterozygotes with associated α-thalassaemia.

The Hb S heterozygotes were grouped into those with one or two α-globin gene deletion, and the values of haematological and biochemical parameters were sepa-rately calculated for each group. The results are presented in Table 6.8. The presence of α-thalassaemia in Hb S heterozygotes modified the haematological parameters although only slightly. The major effect was on the mean cell volume and mean cell haemoglobin, which were both reduced in the group with α-thalassaemia. Individuals with one α-gene deletion had normal total haemoglobin but showed slight microcytosis and hypochromia, while those with those those with homozygαous α-thalasaemia 2 (α/-α) had reduced haemoglobin level and a more pronounced microcytosis and hypochromia. As the number of α-gene deleted increased so did the MCV and MCH. When the Hb S level was correlated with the MCV and MCH, positive correlations were obtained (Figures 6.5 and 6.6). However, some individuals with reduced level of Hb S had normal MCV and MCH as seen in these figures. Similar results were reported in earlier studies (Gelpi, 1971; El-Hazmi, 1986; Al-Awamy et al, 1987).

6.7. Case studies on Saudi Hb S heterozygotes with severe clinical manifestations

During our screening studies in different regions none of the Hb S case complained

of any severe clinical or haematological abnormality, but during the retrospective and prospective studies a number of Hb S heterozygous cases were referred to the King Khalid University Hospital, who were confirmed to have Hb AS on acid and alkaline electrophoresis, but had severe haematological and clinical presentations. Eight were found to have associated β^+-thalassaemia (i.e. they were Hb S β^+-thal. cases), four had α-thalassaemia and five had both α- and β^+-thalassaemia. However, the haematological and clinical presentation in this group of patients was almost as severe as that encountered in the Hb SS cases. Table 6.9 presents the haematological parameters, the α-gene arrangement and ferritin level in each of the 20 cases and Table 6.10 presents the haematological parameter values in the Hb AS grouped according to the associated abnormality.

Differences were encountered in the haematological parameter values and the anaemic state, within these groups, where Hb AS group with α-thalassaemic has the least abnormalities. The red cell morphological data, white blood cells and differential count, and the clinical manifestations in these 20 cases with severe clinical presentation are presented in Tables 6.11 to 6.13. Thus, from these cases it was obvious that despite the asymptomatic nature of Hb S heterozygotes, some complications may result due to associated genetic or acquired abnormalities and though these cases were believed to be Hb AS cases with severe clinical presentation, the occurrance of 2 or more abnormalities in a relatively benign state (i.e. Hb AS) produced severe manifestation. Other unknown factors may have contributed to these complications. Thus, during diagnostic studies it is necessary to take considerable care to reach accurate diagnosis in areas where two or more

genetic abnormalities co-exist in the same individual.

6.8. Care to be taken by Hb S heterozygotes and their physicians

The Hb S heterozygotes are generally assymptomatic, and we did not encounter any major abnormalities except in the twenty cases seen at the hospital, yet going through the literature it was apparent that complications may occur. To avoid any sudden complications certain precautions are necessary and both the patients as well as their physicians should be aware of these. The major precautions to be taken are listed in Table 6.14. With proper care and avoidance of precipitating factor it is almost certain that the Hb S heterozygotes will lead a normal life with no exceptionally major complications.

Table 6.9: Haematological parameters and haemoglobin phenotypes in severely anaemic Hb S heterozygotes

Case No.	Sex/ Age (Yrs)	Haemo-globin pheno-types	Haematological parameters						Hb subtypes (%)				α-gene arrange-ment	Ferritin level	Diagnosis
			Hb (g/dl)	RBC ($x10^{12}$/l)	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	A_2	F	S	A			
29	M 10	AFS	6.9	2.2	0.18	89	32	39	2.8	4.9	48.1	44.2	αα/αα	422	AS/↑Fe
39	M 1	AFS	6.7	2.4	0.18	81	28	37	2.3	9.1	39.6	49.0	-α/-α	226	AS/α-Thal/↑Fe
53	M 10	AFS	9.0	4.1	0.28	73	22	32	4.1	4.3	69.9	21.7	αα/αα	21	AS/β+-Thal
54	M 12	AFS	8.5	2.7	0.23	92	31	36	3.2	4.8	55.2	36.8	αα/αα	107	AS/β+-Thal
85	F 7	AFS	8.4	2.8	0.23	87	30	37	2.7	8.0	68.4	20.9	-α/αα	273	AS/α-Thal/β+/Fe
99	M 5	AFS	7.6	2.6	0.19	80	29	40	2.5	20	54.2	23.3	-α/αα	67	AS/αThalβ+-Thal
103	F 1	AFS	10.0	5.7	0.29	56	18	34	3.2	5.3	42.2	49.3	αα/αα	0.9	AS/Fe deficiency
106	F 1	AS	6.0	1.8	0.15	89	32	40	3.6	3.2	67.1	26.1	-α/αα	68	AS/αThalβ+-Thal
114	M 8	AFS	8.3	2.8	0.22	86	29	37	3.8	5.8	54.2	36.2	-α/αα	205	AS/αThalβ+-Thal
115	M 3	AFS	7.8	2.9	0.21	79	26	36	3.5	5.3	65.7	25.5	-α/αα	215	AS/αThalβ+-Thal
123	F 1	AFS	10.5	3.8	0.24	68	27	42	3.2	4.5	31.6	60.7	-α/αα	5.8	AS/αThal/Fe
126	M 8	AFS	7.8	2.4	0.18	83	33	43	3.3	8.1	38.3	50.3	αα/αα	263	AS/↑Fe
127	M 3	AFS	6.6	1.9	0.15	83	34	45	2.3	9.8	42.1	45.8	αα/αα	252	AS/↑Fe
129	M 10	AFS	8.4	2.3	0.18	86	37	47	3.8	8.7	48.9	38.6	αα/αα	53	AS/β+-Thal
130	F 7	AFS	7.3	2.2	0.16	81	33	45	3.5	10	43.6	42.9	αα/αα	250	AS/β+-Thal/↑Fe
145	M 3	AFS	7.6	2.9	0.20	77	26	37	2.8	7.5	51.3	38.4	αα/αα	196	AS/β+-Thal-thal
146	M 2	AS	9.3	4.5	0.24	59	21	38	3.5	1.5	41.5	53.5	ND	21	AS/α-Thal
150	M 4	AS	11.3	4.0	0.28	75	28	41	3.3	1.8	52.2	42.7	ND	11.5	AS/β+-Thal
163	M 5	AS	8.7	5.3	0.25	51	16	35	2.9	1.1	25.7	70.3	-α/αα	241	AS/α-Thal/↑Fe
169	M 7	AFS	7.9	2.9	0.26	98	28	30	2.7	7.2	61.6	28.5	αα/αα	90	AS/β+-Thal
Normal range	5.2 ± 3.0	AA	13.3 ± 0.86	4.7 ± 0.31	0.38 ± 0.02	81.2 ± 3.3	28.1 ± 1.4	34.6 ± 0.9	2.5 ± 0.5	0.8 ± 0.25	-	-	αα/αα	17-230	Normal

Figure 6.5: Correlation between Hb S and MCH in Saudi Hb S heterozygous

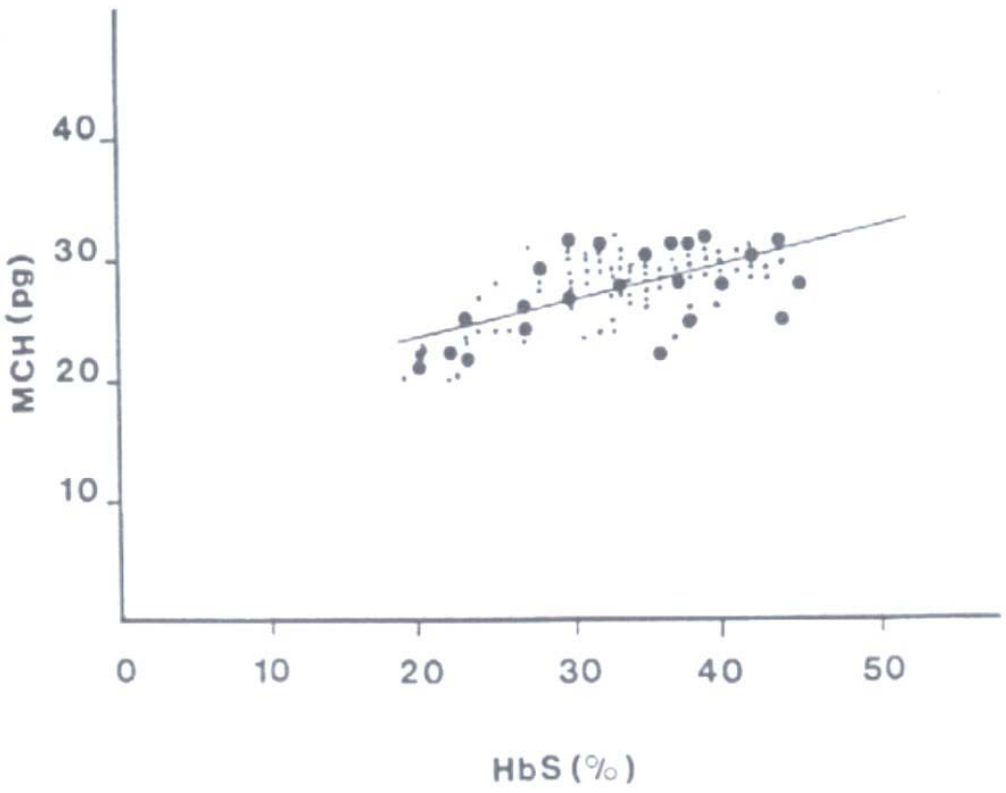


Figure 6.6: Correlation between Hb S and MCV in Saudi Hb S heterozygotes
(● = 1 sample ● = 5 or more samples)

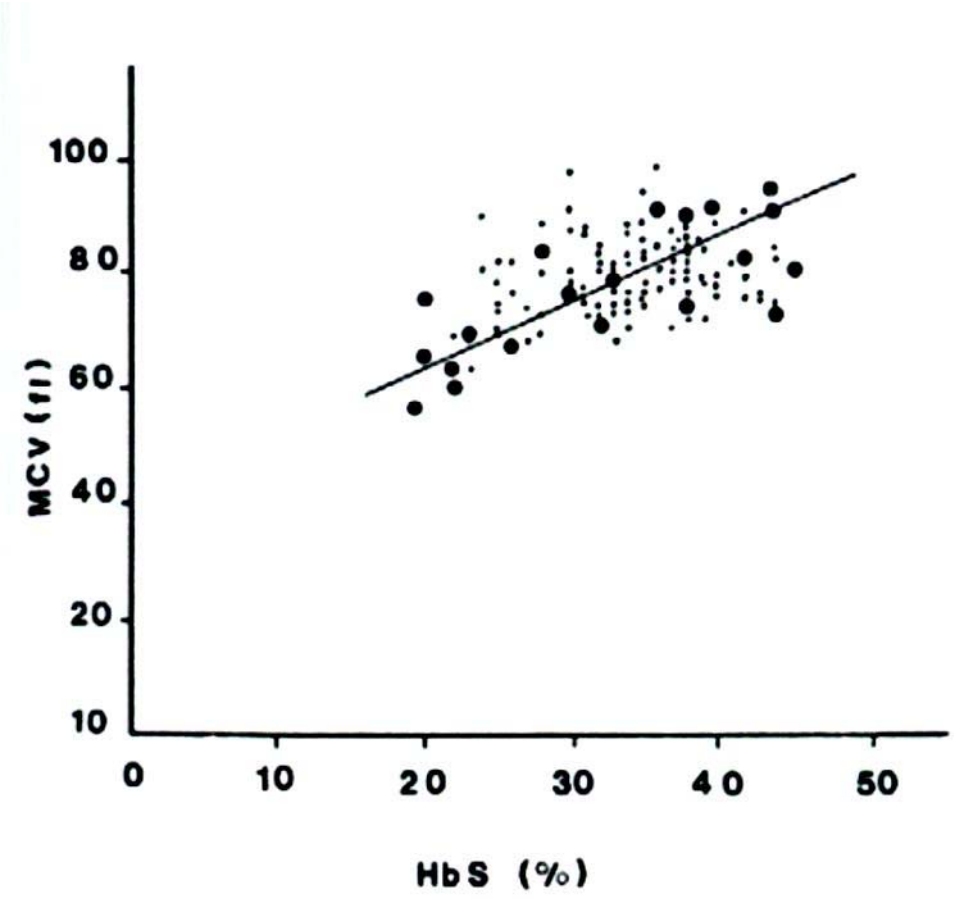


Table 6.10: The value of haematological parameters in Hb S heterozygotes with different associated abnormalities

Group	No.	Hb (g/dl)	RBC $x10^{12}$/l	PCV (l/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Hb A_2 (%)	Hb F (%)	Hb S (%)	Hb A (%)	Ferritin (ng/ml)
AS/↑Fe	3	7.11± 0.624	2.17± 9.252	0.17± 0.017	85.0± 3.5	33.0± 1.0	42.3± 3.0	2.8± 0.5	7.6± 2.5	42.8± 4.94	46.7± 3.1	312.2± 95.0
AS/β+-Thal	7	8.57± 1.33	3.01± 0.76	0.23± 0.05	83.1± 9.3	29.3± 4.9	38.3± 6.3	3.3± 0.5	6.3± 2.8	54.7± 8.7	35.5± 7.7	104.0± 89.0
AS/β+-Thal/ α-Thal	5	7.62± 0.96	2.58± 0.45	0.20± 0.03	84.2± 4.4	29.2± 2.1	38.0± 1.9	3.2± 0.6	8.5± 6.6	61.9± 7.1	26.4± 5.8	165.6± 93.2
AS/β-Thal	4	8.8± 1.58	4.0± 1.2	0.23± 5.6	64.7± 4.8	23.0± 5.6	38.0± 2.9	2.97± 0.51	4.05± 3.7	34.6± 7.3	58.3± 9.3	123.4± 127.4

Table 6.11: Red Cell morphological data in the anaemic Hb S heterozygotes

Case No.	Sickle Cell	Sphero	Poly-chrome	Aniso	Baso-	Poiko	Macro	Target cells	Micro	Hypo-chrome	Hb F cells
29	-	-	+++	++	-	+	+	+	-	-	-
39	-	+	++	++	-	+++	++	-	+	-	+
53	++	+	-	-	-	++	-	+++	+++	++	-
54	-	-	++	++	-	++	+	+	-	-	Occ.
85	+++	+	+	++	+	+	-	+++	-	-	+
99	-	-	-	+	-	+	-	+	-	-	++
103	+	-	-	+	-	++	-	++	++	++	-
106	++++	-	++++	++++	-	+++	++	-	-	-	Occ.
114	+	-	++++	++	-	+	+	++	-	-	Occ.
115	++	+	++++	+++	+	+	++	+	+	+	Occ.
123	++	-	+	-	-	++	-	++	++	+	Occ.
126	+	-	+++	++	-	++	+	+	+	-	++
127	+	+	+++	+++	+	+	+	++	++	-	+
129	++	-	++	++	-	+	+	++	+	-	+
130	+	-	++	+++	-	+	+	+	+	-	+
145	-	-	++	++	-	++	-	+	++	-	+
146	+	-	-	+	-	++	-	+	++	++	-
150	-	-	-	-	-	++	-	+	+	+	-
163	-	-	+	-	-	+++	-	++++	++	++	+++

Table 6.12: White blood cell and differential count in anaemic Hb S heterozygotes

Case No.	WBC (X109/l)	Differential Count				
		Poly (%)	Lymho (%)	Mono (%)	Eisino (%)	Baso (%)
29	24.8	34	53	8	3	-
39	20.7	29	66	5	-	-
53	10.9	51	40	-	8	-
54	21.6	70	22	-	5	-
85	17.9	19	79	1	1	-
99	6.8	51	46	4	-	1
103	12.2	ND	ND	ND	ND	ND
106	14.0	12	86	2	-	-
114	17.0	52	45	1	2	-
115	18.3	32	63	-	5	-
123	10.9	28	65	3	4	-
126	12.7	16	77	-	6	1
127	12.8	28	68	2	2	2
129	14.9	29	59	4	6	2
130	7.4	38	56	3	1	-
145	11.2	ND	ND	ND	ND	ND
146	17.8	19	79	2	-	-
150	5.6	29	62	3	6	-
163	15.5	16	83	1	-	-
169	17.9	ND	ND	ND	ND	ND

Table 6.13: Clinical manifestations in the anaemic Hb S heterozygotes with severe presentations

Case	Jaundice	Pain in bones and joints	Spleno-megaly	Hepato-megaly	Hand & foot Synd-rome	Crisis				No. of blood transfu-sions
						Vaso-occulusive	Infective	Haemolytic	Aplastic	
29	-	-	+	-	-	+	-	-	-	3
39	-	+	+	+	+	+	-	-	-	-
53	-	+	+	-	-	+	-	-	-	1
54	-	+	-	+	-	+	-	-	-	Several
85	-	+	+	-	-	+	-	-	-	2
99	-	+	+	+	-	-	-	-	-	-
103	-	-	-	-	-	-	-	-	-	-
106	-	-	+	+	+	-	-	-	-	-
114	-	-	+	+	-	-	-	-	-	-
115	-	-	+	+	-	-	-	-	-	3
123	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
126	-	-	-	-	-	+	-	+	-	Several
127	-	+	+	-	-	+	-	-	-	1
129	-	+	+	-	-	-	-	-	-	2
130	-	+	+	-	-	-	-	-	-	2
145	-	+	+	-	-	+	-	-	-	2
146	-	-	+	-	-	+	-	-	-	-
150	-	-	+	-	-	-	-	-	-	-
169	-	+	Splenec-tomy	+	+	+	-	-	-	Several

Table 6.14: Care to be taken by Hb S heterozygous cases and by their physicians

1. Avoid extremes of hypoxia
   - Height
   - Underwater swimming
2. Avoid strenous exertion
3. Avoid dehydration
4. General anesthesia must be accompanied by proper oxygenation
5. Avoid deep hypothermia
6. Give partial exchange transfusion during major surgical procedures e.g. Cardiopulmonary bypass.